METHODS FOR IDENTIFYING RISK OF LOW BMD AND TREATMENTS THEREOF

Related Applications

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 60/640,966 filed on 31 December 2004, entitled "Methods For Identifying Risk Of Low BMD And Treatments Thereof," naming Steven Mah et al. as inventors, and designated by attorney docket no. SEQ-4095-PV. This patent application is related to U.S. Provisional Patent Application Nos. 60/640,967 and 60/640,796, each filed on 31 December 2004, entitled "Methods For Identifying Risk Of Low BMD And Treatments Thereof," naming Steven Mah et al. as inventors, and designated by attorney docket nos. SEQ-4094-PV and SEQ-4096-PV, respectively. The content and subject matter of each of these patent applications is hereby incorporated by reference in its entirety, including all text and drawings, in jurisdictions providing for such incorporation.

Field of the Invention

[0002] The invention relates to genetic methods for identifying susceptibility to low bone mineral density (BMD) and/or bone damage generally associated with human diseases, and in particular to osteoporosis, and treatments that specifically target the disease.

Background

[0003] Osteoporosis is a common disease characterized by low bone mineral density (BMD), deterioration of bone micro-architecture and increased risk of bone damage, such as fracture. Common types of osteoporosis include postmenopausal and senile osteoporosis, which generally occur later in life, e.g., 70+ years.

[0004] Osteoporosis is a major public health problem which affects quality of life and increases costs to health care providers. It is estimated that 44 million Americans and 100 million people worldwide are at risk for osteoporosis. In the United States today, 10 million individuals are estimated to already have the disease and almost 34 million more, or 55% of the people 50 years of age and older, have low bone mass, which puts them at increased risk of developing osteoporosis and related fractures. Of the 10 million Americans estimated to have osteoporosis, eight million are women and 2 million are men. These numbers are growing as the elderly population increases. It is estimated that by the middle of the next century the number of osteoporosis sufferers will double in the West, but may increase six-fold in Asia and South America. The estimated national direct expenditures (*e.g.*, hospitals and nursing homes) for osteoporotic and associated fractures was $17 billion in 2001 ($47 million each

day) - and the cost is rising (See National Osteoporosis Foundation; http://www.nof.org/osteoporosis/stats.htm).

[0005] Fracture is the most serious endpoint of osteoporosis, particularly fracture of the hip which affects up to 1.7 million people worldwide each year. One in two women and one in four men over age 50 will have an osteoporosis-related fracture in their lifetime. It is estimated that by the year 2050, the number of hip fractures worldwide will increase to over 6 million, as life expectancy and age of the population increase (See Spangler *et al.* "The Genetic Component of Osteoporosis Mini-review"; http:www.csa.com.osteointro.html).

[0006] Peak bone mass is mainly genetically determined, though dietary factors and physical activity can have positive effects. Peak bone mass occurs when skeletal growth ceases, after which time bone loss starts. In contrast to the positive balance that occurs during growth, in osteoporosis, the resorbed cavity is not completely refilled by bone and BMD decreases. Based on studies of family histories, twin studies, and racial factors, some attribute 50-60% of total bone variation (*e.g.*, Bone Mineral Density to genetic effects and suggest there may be a predisposition for osteoporosis.

[0007] Osteoporosis can be considered a complex genetic trait with variants of several genes underlying the genetic determination of the variability of the phenotype. Low BMD is an important risk factor for fractures, the clinically most relevant feature of osteoporosis. Segregation analysis in families has shown that BMD is under polygenic control. In addition, biochemical markers of bone turnover have shown to have strong genetic components. Several candidate genes have been analyzed in relation to BMD, but the most widely studied gene in this respect, the vitamin D receptor (VDR) gene, explains only a small part of the genetic effect on BMD. Numerous studies, focusing on the *BsmI* allele of the vitamin D receptor gene have concluded that absence of the restriction site correlates with low bone mineral density.

Summary

[0008] It has been discovered that certain polymorphic variations in human genomic DNA are associated with the occurrence of low bone mineral density (BMD) and/or bone damage generally associated with human diseases, and in particular to osteoporosis. In particular, polymorphic variants in loci containing *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1/TNIP1* regions in human genomic DNA have been associated with risk of low BMD.

[0009] Thus, featured herein are methods for identifying a subject at risk of low bone mineral density (BMD) and/or bone fracture, which indicates bone damage and related conditions such as osteoporosis in a subject. The methods comprise detecting the presence or absence of one or more of the polymorphic variations described herein in a human nucleic acid sample. In an embodiment, two or more polymorphic variations are detected and in some embodiments, 3 or more, or 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more polymorphic variants are detected.

[0010] Also featured are nucleic acids that include one or more polymorphic variations associated with occurrence of low BMD, as well as polypeptides encoded by these nucleic acids. In addition, provided are methods for identifying candidate therapeutic molecules for osteoporosis and other low BMD-related disorders, as well as methods for treating osteoporosis in a subject by identifying a subject at risk of low BMD and treating the subject with a suitable prophylactic, treatment or therapeutic molecule.

[0011] Also provided are compositions comprising a cell from a subject suffering from low BMD or at risk of low BMD, and a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1/TNIP1* nucleic acid, with a RNAi, siRNA, antisense DNA or RNA, or ribozyme nucleic acid designed from a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence, or a nucleic acid that hybridizes to such a nucleotide sequence under stringent conditions. In an embodiment, the RNAi, siRNA, antisense DNA or RNA, or ribozyme nucleic acid is designed from a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence that includes one or more low BMD associated polymorphic variations, and in some instances, specifically interacts with such a nucleotide sequence. Further, provided are arrays of nucleic acids bound to a solid surface, in which one or more nucleic acid molecules of the array have a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence, or a fragment or substantially identical nucleic acid thereof, or a complementary nucleic acid of the foregoing. Featured also are compositions comprising a cell from a subject having low BMD or at risk of low BMD and/or a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* polypeptide, with an antibody that specifically binds to the polypeptide. In an embodiment, the antibody specifically binds to an epitope in the polypeptide that includes a non-synonymous amino acid modification associated with low BMD (*e.g.*, results in an amino acid substitution in the encoded polypeptide associated with low BMD). In an embodiment, the antibody specifically binds to an epitope comprising an arginine corresponding to position 120 in a *PROL4* polypeptide (SEQ ID NO: 12).

Brief Description of the Drawings

[0012] Figures 1-4 show the position of each SNP in the chromosome on the x-axis, while the y-axis provides the negative logarithm of the p-value comparing the estimated allele frequency in the cases to that of the control group. Also shown in the figures are exons and introns of the genes in approximate chromosomal positions. More specifically, Figure 1 shows proximal SNPs in a *CETP* region in genomic DNA. Figure 2 shows proximal SNPs in a *PROL4* region in genomic DNA. Figure 3 shows proximal SNPs in a *GRID2* region in genomic DNA. Figure 4 shows proximal SNPs in a *PDE4D* region in genomic DNA. Figure 5 shows proximal SNPs in a *GPX3/TNIP1* region in genomic DNA.

Detailed Description

[0013] It has been discovered that polymorphic variants described in a *CETP, PROL4, GRID2, PDE4D* and *GPX3/TNIP1* loci in human genomic DNA are associated with occurrence of low BMD in subjects. Thus, detecting genetic determinants in and around this locus associated with an increased risk of low BMD occurrence can lead to early identification of a risk of low BMD, or its associated disorders such as osteoporosis, and early application of preventative and treatment measures. Associating the polymorphic variants with low BMD also has provided new targets for diagnosing low BMD, for prognosing osteoporosis, and methods for screening molecules useful in osteoporosis treatments and osteoporosis preventatives.

[0014] Cholestery ester transfer protein (*CETP*) transfers cholesteryl esters between lipoproteins. The transfer of insoluble cholesteryl esters among lipoprotein particles (HDL to LDL) by *CETP* is a step in normal cholesterol homeostasis. The lipoprotein phenotype of *CETP* deficiency, which is characterized by increased levels of HDL and decreased levels of low density lipoprotein (LDL), appears to have antiatherogenic potential. Bone mineral density is reduced by atherogenic diets (increased LDL/HDL ratios), possibly as a result of a shift in the balance of bone marrow stromal cells away from osteoblasts and towards adipocytes, which could result in reduced bone synthesis during remodeling. Lipoproteins may directly regulate bone density via the LRP5, the low-density lipoprotein receptor related protein 5. LRP5 was cloned from an osteoblast cDNA library (Dong, *et al.*: *Biochem Biophys Res Commun.* 1998 Oct 29;251(3):784-90) and mouse knockouts show a reduced BMD phenotype (Kato *et. al.*: *J Cell Biol.* 2002 Apr 15;157(2):303-14). A G171V mutation in LRP5 resulted in increased numbers of osteoblasts, increased AP activity, and increased trabecular number and thickness in mice (Babij *et al.*: *J Bone Miner Res.* 2003 Jun; 18(6): 960-74). The human G171V mutation was found to have the same phenotype, presumably by increasing WNT signaling in osteoblasts (Mao *et. al.*: *Mol Cell.* 2001 Apr;7(4):801-9). Overall, there is evidence that lipoprotein profiles play a role in osteoblast differentiation. Provided is a method for treating osteoporosis or low bone mineral density by modulating a *CETP* function in a subject suffering from osteoporosis or low bone mineral density. *CETP* inhibitors and methods of making them are described in US 6,586,613 (Substituted tetrahydronaphthaline and analogous compounds); US 6,562,976 (4-phenyltetrahydrochinoline utilized as an inhibitor of the cholesterol ester transfer protein); US 6,387,929 (4-heteroaryl-tetrahydroquinolines and their use as inhibitors of the cholesterin-ester transfer protein); US 6,291,477 (Tetrahydroquinolines, processes for their preparation, pharmaceutical compositions containing them, and their use to prevent or treat hyperlipoproteinaemia); US 6,218,431 (Substituted biphenyls); US 6,207,671 (Cycloalkano-pyridines); US 6,127,383 (2-aryl-substituted pyridines); US 6,121,330 (5-Hydroxyalkyl substituted phenyls and their use in medicaments for the treatment of arteriosclerosis and hyperlipoproteinaemia); US 6,069,148 (Cycloalkano-pyridines); US 6,063,788 (Bicyclic-fused pyridines); US 5,932,587 (Heterocyclic-fused pyridines); US 5,925,645 (2-aryl-substituted pyridines); US 6,753,346 (CETP activity inhibitor); US 6,706,881 (Methods for

preparing CETP inhibitors); US 6,689,897 (Intermediates of CETP inhibitors); US 6,600,045 (Methods for preparing CETP inhibitors); US 6,573,383 (Preparation of anhydrous CETP inhibitor); US 6,555,113 (Modulation of cholesteryl ester transfer protein (CETP) activity); US 6,426,365 (CETP activity inhibitors); US 6,410,022 (Modulation of cholesteryl ester transfer protein (CETP) activity); US 6,410,020 (Monoclonal antibody reactive to human CETP and assay method for human CETP); US 6,140,474 (Monoclonal antibody reactive with human-origin CETP and method of quantifying human-origin CETP); US 5,948,435 (Methods of regulating CETP genes, enzymes and other compound, and pharmaceutical composition therefor); US 5,519,001 (CETP inhibitor polypeptide antibodies against the synthetic polypeptide and prophylactic and therapeutic anti-atherosclerosis treatments); and US 5,512,548 (CETP inhibitor polypeptide, antibodies against the synthetic polypeptide and prophylactic and therapeutic anti-atherosclerosis treatments).

[0015] *PROL4*, also known as Lacrimal proline rich protein (LPRP) is a member of the proline-rich secreted protein family and contains a conserved acidic N-terminal region. It has 45.5% amino acid homology to a salivary Parotid acidic protein (PRH1). PRH1 and related proline-rich (salivary) proteins act as potent inhibitors of hydroxyapatite crystal growth and bind calcium with a strength that suggests that they are important in maintaining the concentration of ionic calcium in saliva. The N-terminal region of salivary proline-rich proteins, which is also the region of strongest homology to *PROL4*, mediates this binding. It is expected that *PROL4* plays a role in maintaining calcium levels during bone remodeling, and that a loss of function of *PROL4* facilitates bone loss. Provided is a method of treating low BMD or osteoporosis by increasing expression of *PROL4* or otherwise increasing levels of active *PROL4* in a patient suffering from low BMD or osteoporosis.

[0016] Human glutamate receptor delta-2 (*GRID2*) is a member of the family of ionotropic glutamate receptors that are excitatory neurotransmitter receptors in mammalian brain. A point mutation in mouse *GRID2*, associated with the phenotype named "lurcher" in the heterozygous state, leads to ataxia resulting from selective, cell-autonomous apoptosis of cerebellar Purkinje cells during postnatal development. Mice homozygous for this mutation die shortly after birth from massive loss of mid- and hindbrain neurons during late embryogenesis. Glutamate receptors have been detected in osteoblasts and osteoclasts and may regulate bone resorption. Provided is a method of treating low bone mineral density or osteoporosis by modulating *GRID2* function in a patient suffering from low BMD or osteoporosis.

[0017] *PDE4D* encodes cyclic AMP-dependent phosphodiesterase 4D. Phosphodiesterases are a superfamily of enzymes involved in degradation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (Manganiello VC, *et al.*: *Arch Biochem Biophys* 1995, 322(1):1-13; and Beavo JA: *Physiol Rev* 1995, 75(4):725-748). cAMP and cGMP are important second messengers participating in the response of various cells to hormones. In osteoblasts, cAMP produced in response to parathyroid hormone or prostaglandins regulates osteoblastic differentiation (Farndale RW, *et al. Biochem J* 1988, 252(1):263-268; Kumegawa M, et al. *Calcif Tissue Int* 1984, 36(1):72-76;

Ishizuya T, *et al. J Clin Invest* 1997, 99(12):2961-2970; and Partridge NC, *et al. J Cell Biochem* 1994, 55(3):321-327), which leads to increases in cancellous bone volume as indicated by experiments in animal models (Jee WS, *et al. Bone* 1987, 8(3):171-178; High WB: et al.. *Bone* 1987, 8(6):363-373; Reeve J: et al. *J Bone Miner Res* 1996, 11(4):440-445; and Finkelstein JS, et al. *N Engl J Med* 1994, 331(24):1618-1623). Intracellular levels of cAMP are regulated by G protein-coupled adenylyl cyclase (Casperson GF, Bourne HR: *Annu Rev Pharmacol Toxicol* 1987, 27:371-384), and degradation is mediated by the phosphodiesterases. The phosphodiesterase superfamily consists of seven families, PDE1-7, distinguished by substrate specificity, chromatographic behavior during purification, and affinity for biochemical activators and inhibitors. Of these, the PDE4 family is specific for cAMP and is selectively inhibited by rolipram. Four PDE4 genes, 4A, 4B, 4C, and 4D, have been cloned from rat and humans, all of which are predicted to have multiple protein products due to alternate spicing of RNAs. PDE4 inhibitors have been shown to increase bone formation in normal mice (Kinoshita T, et al. *Bone* 2000, 27(6):811-817) and to ameliorate loss of bone mass in animal models of osteopenia (Miyamoto K, et al. *Biochem Pharmacol* 1997, 54(5):613-617; and Waki Y, et al. *Jpn J Pharmacol* 1999, 79(4):477-483). PDE4A and PDE4D are expressed in two common mouse osteoblastic cell lines, ST2 and MC3T3-E1, that represent different stages in the osteoblast differentiation pathway (Wakabayashi S, et al.. *J Bone Miner Res* 2002, 17(2):249-256). PDE4 inhibition with rolipram increased BMP2-induced alkaline phosphatase activity, a marker of early osteoblast differentiation in ST2 cells. Furthermore, rolipram increased the expression of alkalic phosphatase, osteopontin, collagen type I and osteocalcin in the same osteoblast precursor cells (Wakabayashi S, et al. *J Bone Miner Res* 2002, 17(2):249-256).

[0018] Provided herein is a method for treating osteoporosis or low bone mineral density by modulating a *PDE4* function in a human suffering from osteoporosis or low bone mineral density. Modulation of PDE4D by PDE4D inhibitors may increase bone formation and treat an osteoporotic condition. PDE4D inhibitors and methods of making them are described in the following US patents: 6,218,400 (Treatment method using a cGMP-Specific PDE inhibitor), 5,891,896 (Tri-substituted phenyl derivatives useful as PDE IV inhibitors), 5,849,770 (Tri-substituted phenyl derivatives useful as PDE IV inhibitors), 5,712,298 (Fluoroalkoxy-substituted benzamides and their use as cyclic nucleotide phosphodiesterase inhibitors), 5,491,147 (Tri-substituted phenyl derivatives and their use in pharmaceutical compositions and methods of treatment). Also provided is a method of treating osteoporosis by decreasing expression of PDE4D or otherwise decreasing levels of active PDE4D in a subject suffering from low BMD. Provided also is a method of targeting pertinent information or administering preventative or therapeutic treatments to a subject based on a subject's PDE4D genotype.

[0019] SNP rs869975 is contained within the *GPX3* gene, and the *TNIP3* gene may not be ruled out due to linkage disequilibrium; therefore the *GPX3/TNIP3* region is considered associated with low BMD. The *GPX3* gene encodes the Plasma glutathione peroxidase 3 precursor. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide, organic hydroperoxide, and lipid peroxides by

reduced glutathione and functions in the protection of cells against oxidative damage. This enzyme, found mainly in the cytosol of mammalian cells, is unusual in its content of a selenocysteine residue in its active site that is encoded by a TGA opal codon. Selenium deficiency causes bone loss and might contribute to lower BMD. Osteoblasts produce glutathione peroxidase, possibly as a defense against hydrogen peroxide produced by osteoclasts during bone remodeling and thus may contribute to lower BMD. Provided is a method for treating osteoporosis or low bone mineral density by modulating a *GPX* function in a human suffering from osteoporosis or low bone mineral density.

[0020] In the TNIP1/NAF1/ABIN-1 pathway, NAF1 was identified by a yeast two-hybrid screen as an interacting protein to the HIV protein, Nef. Subsequently, it was found to be an A20-binding protein that is critical for the A20-mediated negative feedback regulation of NF-kappa B activation in response to tumor necrosis factor (TNF). As TNF is a critical effector of the pathogenesis of rheumatoid arthritis (RA), Gallagher *et. al.* (*FEBS Lett.* 2003 Sep 11;551(1-3):8-12), tested TNF-alpha-modulated gene expression in cultured primary human synoviocytes *in vitro*. Genes upregulated included *TNIP1* and implicate *TNIP1* as a potential modulator of TNF-alpha bioactivity in RA. The differentiation and functions of osteoclasts are stimulated and regulated by osteoblast/stromal cell derived factors, such as receptor activator of NFKB ligand (RANKL). Provided is a method for treating osteoporosis or low bone mineral density by modulating a *TINIP* function in a human suffering from osteoporosis or low bone mineral density..

Low BMD and Sample Selection

[0021] The present invention is applicable to any disease in which low BMD and/or bone fracture is a factor, and is therefore particularly concerned with diseases such as osteoporosis. Low BMD is defined by the World Health Organization as 2.5 standard deviations below the age-matched mean of bone mineral density for a given population. Bone damage may be defined as any form of structural damage such as fractures or chips of the bone, and degradation or deterioration of the bone other than normal wear and tear resulting from low bone mineral density or another cause. Such low BMD and/or bone damage is associated with osteoporosis.

Osteoporosis, or porous bone, is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures, especially of the hip, spine and wrist. In general, there are two types of osteoporosis: primary and secondary. Approximately 90% of all osteoporosis cases is idiopathic "primary osteoporosis". Such primary osteoporosis includes postmenopausal osteoporosis, age-associated osteoporosis (affecting a majority of individuals over the age of 70 to 80), and idiopathic osteoporosis affecting middle-aged and younger men and women. "Secondary osteoporosis" is the result of an identifiable disease process or agent.

[0022] For some osteoporotic individuals, the loss of bone tissue is sufficiently great so as to cause mechanical failure of the bone structure. Bone fractures often occur, for example, in the hip and spine

of women suffering from postmenopausal osteoporosis. Kyphosis (abnormally increased curvature of the thoracic spine) may also result.

[0023] The mechanism of bone loss in osteoporotics is believed to involve an imbalance in the process of "bone remodeling". Bone remodeling occurs throughout life, renewing the skeleton and maintaining the strength of bone. This remodeling involves the erosion and filling of discrete sites on the surface of bones, by an organized group of cells called "basic multicellular units" or "BMUs". BMUs primarily consist of "osteoclasts", "osteoblasts", and their cellular precursors. In the remodeling cycle, bone is resorbed at the site of an "activated" BMU by an osteoclast, forming a resorption cavity. This cavity is then filled with bone by an osteoblast.

[0024] Normally, in adults, the remodeling cycle results in a small deficit in bone, due to incomplete filling of the resorption cavity. Thus, even in healthy adults, age-related bone loss occurs. However, in osteoporotics, there is an increase in the number of BMUs that are activated. This increased activation accelerates bone remodeling, resulting in abnormally high bone loss.

[0025] Preferred methods for the treatment of osteoporosis include an initial diagnostic step to determine the presence of the disorder. Initial diagnostic steps include determination of bone mass and rate of bone remodeling. The rate of bone remodeling can be determined by the measurement of biochemical markers. See, for example, Hui *et al.*, "The Contribution of Bone Loss to Postmenopausal Osteoporosis" *Osteoporosis Int.* 30 (1990). Diagnosis of those at risk of developing osteoporosis also allows more effective preventive measures. Part of diagnosis includes specialized tests called bone density tests that measure bone density in various sites of the body. Such methods include the measurement of the radiodensity of skeletal radiographs, quantitative computerized tomography, single energy photon absorptiometry, and dual-energy photon absorptiometry. Diagnostic techniques among those useful herein are described in W. A. Peck *et al.*, Physician's Resource Manual on Osteoporosis (1987), published by the National Osteoporosis Foundation. A bone density test can detect the presence of low BMD before a fracture occurs, predict your chances of fracturing in the future, determine rate of bone loss, and monitor response to treatment.

[0026] Based in part upon selection criteria set forth above, individuals having low BMD can be selected for genetic studies. Also, individuals having a family history of low BMD or diagnosed with osteoporosis often are selected for genetic studies. Other selection criteria can include: a tissue or fluid sample derived from an individual characterized as Caucasian; sample derived from an individual of Caucasian paternal and maternal descent; case samples derived from individuals diagnosed with osteoporosis; control samples derived from individuals with normal or high BMD levels and no family history of osteoporosis; and sufficient genomic DNA for all allelotyping and genotyping reactions performed during the study. Phenotype information may included pre- or post-menopausal, familial predisposition, country or origin of mother and father, diagnosis with osteoporosis (date of primary diagnosis, age of individual as of primary diagnosis, osteoporosis-related fracture), biochemical measurements of markers of bone resorption (bone-specific alkaline phosphatase, urinary C-telopeptide

of type I collagen, serum osteocalcin), current medication status (thyroid medication, hormone replacement therapy, steroid usage, bisphosphonates and cytotoxic agents for rheumatic diseases). Samples that meet the inclusion criteria and do not meet the exclusion criteria may be added to appropriate pools based on gender and disease status.

Polymorphic Variants Associated with Low BMD

[0027] A genetic analysis provided herein linked low BMD with polymorphic variant nucleic acid sequences in the human genome. As used herein, the term "polymorphic site" refers to a region in a nucleic acid at which two or more alternative nucleotide sequences are observed in a significant number of nucleic acid samples from a population of individuals. A polymorphic site may be a nucleotide sequence of two or more nucleotides, an inserted nucleotide or nucleotide sequence, a deleted nucleotide or nucleotide sequence, or a microsatellite, for example. A polymorphic site that is two or more nucleotides in length may be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more, 20 or more, 30 or more, 50 or more, 75 or more, 100 or more, 500 or more, or about 1000 nucleotides in length, where all or some of the nucleotide sequences differ within the region. A polymorphic site is often one nucleotide in length, which is referred to herein as a "single nucleotide polymorphism" or a "SNP."

[0028] Where there are two, three, or four alternative nucleotide sequences at a polymorphic site, each nucleotide sequence is referred to as a "polymorphic variant" or "nucleic acid variant." Where two polymorphic variants exist, for example, the polymorphic variant represented in a minority of samples from a population is sometimes referred to as a "minor allele" and the polymorphic variant that is more prevalently represented is sometimes referred to as a "major allele." Many organisms possess a copy of each chromosome (*e.g.*, humans), and those individuals who possess two major alleles or two minor alleles are often referred to as being "homozygous" with respect to the polymorphism, and those individuals who possess one major allele and one minor allele are normally referred to as being "heterozygous" with respect to the polymorphism. Individuals who are homozygous with respect to one allele are sometimes predisposed to a different phenotype as compared to individuals who are heterozygous or homozygous with respect to another allele.

[0029] In genetic analysis that associate polymorphic variants with low BMD, samples from individuals having low BMD and individuals not having low BMD often are allelotyped and/or genotyped. The term "allelotype" as used herein refers to a process for determining the allele frequency for a polymorphic variant in pooled DNA samples from cases and controls. By pooling DNA from each group, an allele frequency for each SNP in each group is calculated. These allele frequencies are then compared to one another. The term "genotyped" as used herein refers to a process for determining a genotype of one or more individuals, where a "genotype" is a representation of one or more polymorphic variants in a population.

[0030] A genotype or polymorphic variant may be expressed in terms of a "haplotype," which as used herein refers to two or more polymorphic variants occurring within genomic DNA in a group of

individuals within a population. For example, two SNPs may exist within a gene where each SNP position includes a cytosine variation and an adenine variation. Certain individuals in a population may carry one allele (heterozygous) or two alleles (homozygous) having the gene with a cytosine at each SNP position. As the two cytosines corresponding to each SNP in the gene travel together on one or both alleles in these individuals, the individuals can be characterized as having a cytosine/cytosine haplotype with respect to the two SNPs in the gene.

[0031] As used herein, the term "phenotype" refers to a trait which can be compared between individuals, such as presence or absence of a condition, a visually observable difference in appearance between individuals, metabolic variations, physiological variations, variations in the function of biological molecules, and the like. An example of a phenotype is occurrence of low BMD or clinically diagnosed osteoporosis.

[0032] Researchers sometimes report a polymorphic variant in a database without determining whether the variant is represented in a significant fraction of a population. Because a subset of these reported polymorphic variants are not represented in a statistically significant portion of the population, some of them are sequencing errors and/or not biologically relevant. Thus, it is often not known whether a reported polymorphic variant is statistically significant or biologically relevant until the presence of the variant is detected in a population of individuals and the frequency of the variant is determined. Methods for detecting a polymorphic variant in a population are described herein, specifically in Example 2. A polymorphic variant is statistically significant and often biologically relevant if it is represented in 5% or more of a population, sometimes 10% or more, 15% or more, or 20% or more of a population, and often 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, or 50% or more of a population.

[0033] A polymorphic variant may be detected on either or both strands of a double-stranded nucleic acid. Also, a polymorphic variant may be located within an intron or exon of a gene or within a portion of a regulatory region such as a promoter, a 5′ untranslated region (UTR), a 3′ UTR, and in DNA (*e.g.*, genomic DNA (gDNA) and complementary DNA (cDNA)), RNA (*e.g.*, mRNA, tRNA, and rRNA), or a polypeptide. Polymorphic variations may or may not result in detectable differences in gene expression, polypeptide structure, or polypeptide function.

[0034] It was determined that polymorphic variations associated with an increased risk of low BMD existed in *CETP, PROL4, GRID2, PDE4D* and *GPX3* nucleotide sequences. In the *CETP* locus, polymorphic variants at positions selected from the group consisting of rs7500979, rs2217332, rs8044804, rs2270835, rs2133783, rs247609, rs952440, rs881598, rs2291955, rs2518054, rs866038, rs1436425, rs173537, rs247611, rs166017, rs173538, rs193694, rs7205692, rs8048746, rs247618, rs183130, rs6499863, rs4783961, rs3816117, rs711752, rs708272, rs1864163, rs4369653, rs1864165, rs891141, rs891143, rs7205804, rs5885, rs1532625, rs1532624, rs289712, rs7499892, rs5883, rs289714, rs158480, rs289717, rs4344729, rs289718, rs289719, rs2033254, rs4784744, rs291044, rs8053613, rs5881, rs5880, rs7198026, rs5882, rs8045701, rs289741, rs1801706, rs289742, rs289743,

rs289746, rs172337, rs289747, rs1566439, rs7205459, rs289749, rs289751, rs8059220, rs8058353, rs289735, rs289737, rs291042, rs1875236, rs821466, rs821465, rs4275846, rs289707, rs821463, rs289706, rs1167741, rs2052880, rs1167742, rs1183256, rs1651665, rs1651666, rs4784751, rs1651667, rs8052091, rs1684574, rs1684575, rs1672865, rs821470, rs1549669, rs291040 and rs289754 were tested for association with low BMD. Polymorphic variants at the following positions were associated with low BMD: rs166017, rs193694, rs7205804, rs1801706, rs7205459 and rs821465. At these positions in SEQ ID NO:1, a thymine at position 14328, a thymine at position 14996, a guanine at position 37336, a guanine at position 50109, a thymine at position 57618, and a guanine at position 68805 were associated with low BMD.

[0035] In the *PROL4* locus, polymorphic variants at positions selected from the group consisting of rs523051, rs693620, rs2588349, rs2588350, rs619381, rs3759252, rs3759251, rs2418107, rs7303054, rs1838345, rs620878, rs2537817, rs1548803, rs667123, rs1838346, rs2159903, rs3944035, rs3741845, rs2110096, rs759055, rs589377, rs7960194, rs7978242, rs601051, rs4262797, rs2215714, rs1373434, rs2215715, rs612456, rs612808, rs689118, rs597468, rs592864, rs640372, rs7966559, rs654834, rs4763216, rs668521, rs669503, rs3906864, rs3906863, rs7957888, rs9300230, rs7306214, rs763839, rs2418105, rs666841, rs3851578, rs7138797, rs7295252, rs2418106, rs7299578, rs621112, rs3863320, rs1373432, rs1047699, rs1063193, rs2232959, rs2227296, rs1548804, rs2232958, rs2232957, rs2232956, rs1972571, rs3759250, rs3759249, rs1541525, rs2098248, rs2900550, rs7302130, rs4763583, rs4360778, rs1607695, rs1607694, rs2192139, rs7978300, rs7397871, rs4763217, rs2159900, rs10772370, rs7398682, rs2900551, rs2900552, rs2418214, rs2418215, rs965243, rs1117548, rs1520225, rs1520226, rs1520227, rs971919, rs2159901, rs2159902, rs2110099, rs7314847, rs7296003, rs4281556, rs4763219, rs3851579, rs3851580, rs1049119, rs2298866, rs2298865, rs2298864, rs2298863, rs3180393, rs2070837, rs7956204, rs2418216, rs3741844, rs4262798, rs2418217, rs2418218, rs7137492, rs2110100, rs1013312, rs4579993, rs1013313, rs7397106, rs2215716, rs2192140, rs4763589, rs1468697, rs2070837, rs3180393 and rs2298865 were tested for association with low BMD. Polymorphic variants at the following positions were associated with an increased risk of low BMD: rs2588350, rs619381, rs620878, rs759055, rs4262797, rs612808, rs3906863, rs7957888, rs763839, rs2418105, rs666841, rs3851578, rs7299578, rs621112, rs1047699, rs1548804, rs2232956, rs1520227 and rs2215716. At these positions in SEQ ID NO:2, a cytosine at position 2424, a cytosine at position 3625, a guanine at position 7097, an adenine at position 15688, a guanine at position 22861, a cytosine at position 24138, a cytosine at position 32459, an adenine at position 35151, a guanine at position 36930, an adenine at position 37490, a cytosine at position 38432, an adenine at position 38688, a guanine at position 42665, an adenine at position 43038, a cytosine at position 49075, an adenine at position 50773, an adenine at position 52107, a cytosine at position 75246, and a guanine at position 93715 were associated with risk of low BMD.

[0036] In the *GRID2* locus, polymorphic variants at positions selected from the group consisting of rs1433661, rs1485009, rs7681947, rs1816432, rs1485018, rs1485017, rs7438397, rs6834311,

rs1368717, rs1017391, rs2870701, rs7679839, rs1385404, rs1368716, rs4693316, rs1905707, rs1905708, rs1905709, rs3912442, rs2082553, rs6831638, rs5860329, rs2870702, rs2870703, rs1948016, rs6835836, rs1994253, rs1905710, rs1485019, rs978191, rs1385405, rs7694361, rs1905711, rs1905734, rs1485012, rs1485013, rs4692981, rs7670552, rs7670932, rs7688091, rs7440540, rs2171000, rs2870704, rs7655758, rs7661436, rs7662289, rs7667044, rs7691929, rs5860330, rs901013, rs901012, rs901011, rs1948018, rs2870705, rs1948017, rs1905733, rs1385408, rs1385409, rs1385410, rs1485026, rs1485027, rs2904483, rs1385406, rs1905732, rs2046418, rs2200377, rs1905731, rs1905730, rs975713, rs6820985, rs7670441, rs6810794, rs7676623, rs1154861, rs1032125, rs1485022, rs1485024, rs3913651, rs4693319, rs1872383, rs2200376, rs7668090, rs7692930, rs967096, rs6822249, rs6532405, rs1017897, rs7672674, rs7694568, rs2904484, rs7340830, rs1485033, rs2870706, rs1905729, rs4693320, rs6848749, rs6532406, rs6532407, rs1905728, rs6819866, rs1905727, rs7674069, rs1905724, rs1905723, rs1485020 and rs6814101 were tested for association with low BMD. Polymorphic variants at the following positions were associated with an increased risk of low BMD: rs1433661, rs7679839, rs1368716, rs1905707, rs1905708, rs1994253, rs1485019, rs1905734, rs1485012, rs7670552, rs7691929, rs1948018, rs1948017, rs1485024, rs7694568, rs4693320, rs6848749, rs6532406, rs6532407 and rs6819866. At these positions in SEQ ID NO:3, a thymine at position 206, a guanine at position 8612, an adenine at position 9285, a thymine at position 11866, a guanine at position 11958, a cytosine at position 28773, a thymine at position 29876, a cytosine at position 35588, a guanine at position 37663, a thymine at position 39375, a cytosine at position 43705, an adenine at position 48962, a cytosine at position 49110, an adenine at position 65050, a cytosine at position 78331, a thymine at position 85405, a guanine at position 86441, an adenine at position 86967, a cytosine at position 87121, and an adenine at position 90969 were associated with risk of low BMD.

[0037] In the *PDE4D* locus, polymorphic variants at positions selected from the group consisting of rs6886495, rs6450498, rs1472456, rs4700315, rs4700316, rs7714708, rs7710479, rs2968013, rs2968014, rs2968015, rs1391648, rs2055297, rs2055296, rs3989138, rs4700317, rs2036220, rs7727206, rs7723432, rs1546221, rs4479801, rs4395595, rs4395596, rs4699932, rs2936201, rs7356672, rs2936200, rs1909296, rs7703131, rs7445308, rs3087748, rs4321723, rs2968016, rs5868151, rs1874858, rs1874857, rs7712922, rs4631140, rs4469166, rs1078369, rs1078368, rs2968006, rs2968005, rs2936190, rs2409613, rs4415048, rs2968004, rs2968003, rs2968002, rs2936191, rs1498610, rs6874662, rs3060393, rs7729722, rs7733884, rs7714489, rs7735570, rs2936193, rs2291851, rs2291852, rs1498602, rs1995166, rs1498603, rs1498604, rs1498605, rs1948651, rs4699934, rs4700319, rs2279737, rs7720361, rs7706419, rs1006431, rs1353747, rs1498606, rs1353748, rs1553113, rs2968012, rs2968011, rs1498608, rs2936189, rs1498609, rs2968019, rs6891238, rs2968010, rs2968009, rs2936203, rs1498601, rs1498600, rs1498599, rs2936202, rs7730070, rs6450501, rs6450502, rs6889456, rs6894618, rs7706044, rs7707541, rs7712076, rs6892860, rs6867053, rs7737269, rs6864156, rs950447, rs2936196, rs7719347,

rs1391649, rs1391650, rs1391651, rs1353749, rs10682149, rs5868153, rs1363882, rs2409626, rs2968018, rs954740, rs986067, rs6869400, and rs5010782 were tested for association with low BMD. Polymorphic variants at the following positions were associated with an increased risk of low BMD: rs7714708, rs1498602, rs4699934, rs1006431, rs1353747, rs1498608, rs1498609, rs2968010, rs2936202 and rs1391649. At these positions in SEQ ID NO:4, an adenine at position 1599, a cytosine at position 39626, a thymine at position 40356, a thymine at position 43555, a thymine at position 44066, a thymine at position 49652, a cytosine at position 51103, an adenine at position 57173, a guanine at position 63980, and an adenine at position 82591 were associated with risk of low BMD.

[0038] In the *GPX3/TNIP1* locus, polymorphic variants at positions selected from the group consisting of rs1478398, rs1478397, rs1160114, rs1160113, rs1382323, rs1160112, rs7709870, rs7710643, rs7730467, rs6579829, rs6579830, rs6579831, rs6896232, rs1351131, rs1038074, rs1478396, rs6880512, rs4958858, rs4958431, rs4958432, rs6898463, rs4958859, rs4130064, rs4130065, rs4133119, rs4958860, rs4958861, rs4437356, rs4958868, rs1478400, rs6889375, rs1600159, rs6875892, rs4608909, rs2345000, rs4516840, rs2054440, rs707141, rs707142, rs841236, rs707143, rs707144, rs6869405, rs707145, rs707146, rs707148, rs707150, rs5872184, rs3763015, rs2042235, rs3763013, rs2042236, rs1946234, rs1946235, rs1946236, rs8177402, rs8177403, rs8177404, rs8177405, rs8177406, rs8177407, rs8177408, rs8177409, rs6888961, rs8177410, rs8177411, rs8177412, rs8177413, rs870407, rs870406, rs6873202, rs8177414, rs8177415, rs3805435, rs8177416, rs3792799, rs3792798, rs3828599, rs8177417, rs3792797, rs8177418, rs8177419, rs8177420, rs8177421, rs4958872, rs3792796, rs8177422, rs8177423, rs4958434, rs8177424, rs8177425, rs8177426, rs8177427, rs8177429, rs6889737, rs3792795, rs8177430, rs8177431, rs4958873, rs8177432, rs8177433, rs8177434, rs8177435, rs3763011, rs8177436, rs8177437, rs4958874, rs8177439, rs8177440, rs8177441, rs8177442, rs8177443, rs869975, rs869976, rs8177444, rs8177445, rs7721469, rs8177446, rs7704191, rs8177447, rs11548, rs2230303, rs7722386, rs8177448, rs8177449, rs2070593, rs8177450, rs8177451, rs8177452, rs8177453, rs8177454, rs3763010, rs8177455, rs8177456, rs736775, rs2277940, rs8177458, rs8177834, rs3924, rs2233312, rs2233311, rs2233310, rs2233309, rs4958875, rs2233308, rs2233307, rs2233306, rs2233305, rs2233304, rs2233303, rs2233302, rs2287719, rs2287720, rs7727034, rs7727250, rs7709800, rs3840312, rs2287721, rs6875293, rs3805434, rs2080982, rs2080983, rs2287722, rs2233301, rs2233300, rs4958876, rs2233299, rs2233298, rs2287723, rs2161359, rs7734456, rs4292439, rs4958878, rs6862024, rs3834819, rs2233297, rs2233296, rs2233295, rs2233294, rs7713028, rs7713223, rs7713567, rs888989, rs2233293, rs3749657, rs2233292, rs2112635, rs871269, rs3792794, rs6579837, rs3805433, rs5872186, rs2233291, rs2233290, rs2233289, rs4958435, rs4958880, rs1422673, rs2042234, rs3805432, rs3805431, rs2233288, rs2233287, rs3815720, rs3792792, rs3792791, rs2303018, rs3792790, rs4958436, rs2233286, rs2233285, rs7732451, rs2233284, rs1422674, rs3792789, rs4562032, rs6865077, rs1559126, rs3792788, rs1559127, rs3792786, rs6880110, rs6861227, rs3805430, rs1862364, rs4958881, rs3792785, rs6869605, rs6870205, rs4246047,

rs4958882, rs3792784, rs3792783 and rs5872188 were tested for association with low BMD. Polymorphic variants at the following positions were associated with an increased risk of low BMD: rs1478398, rs1160114, rs1160113, rs1160112, rs4958858, rs4958431, rs6898463, rs4958859, rs4958860, rs4608909, rs707144, rs2042235, rs3763013, rs2042236, rs8177404, rs8177426, rs8177427, rs8177429, rs3792795, rs4958873, rs8177437, rs869975, rs8177447, rs11548, rs2277940, rs8177834, rs2233311, rs2233302, rs7727034, rs7727250, rs3805434, rs7734456, rs7713028, rs7713223, rs888989, rs3792794, rs4958880, rs1422673, rs3805432 and rs4958436. At these positions in SEQ ID NO:5, an adenine at position 231, a cytosine at position 582, a guanine at position 589, an adenine at position 1066, a thymine at position 5621, a guanine at position 5735, a cytosine at position 6658, a cytosine at position 7901, a thymine at position 15803, a cytosine at position 25599, a thymine at position 31203, a thymine at position 41624, a guanine at position 41671, an adenine at position 41825, a cytosine at position 43294, an adenine at position 46650, an adenine at position 46721, a guanine at position 46808, a guanine at position 47512, an adenine at position 47806, a guanine at position 49097, a guanine at position 50082, a thymine at position 51166, a cytosine at position 51493, a thymine at position 53187, an adenine at position 53699, a thymine at position 53929, a cytosine at position 58808, a cytosine at position 59187, a cytosine at position 59361, a cytosine at position 64049, a guanine at position 70882, an adenine at position 74131, a cytosine at position 74406, a cytosine at position 74740, a guanine at position 78432, a cytosine at position 82187, a cytosine at position 82698, an adenine at position 83214 and a thymine at position 86539 were associated with risk of low BMD.

[0039] Based in part upon analyses summarized in Figures 1-5, regions with significant association have been identified in loci associated with low BMD. Any polymorphic variants associated with low BMD in a region of significant association can be utilized for embodiments described herein. The following table reports such regions, where "begin" and "end" designate the boundaries of the region according to chromosome positions within the genomic sequence provided in SEQ ID Nos:1-5. The locus, the chromosome on which the locus resides and an incident polymorphism in the locus also are noted.

TABLE 1: COMBINED ASSOCIATION RANGES

Incident SNP	Locus	Chromosome	Begin	End	Size
rs1801706	CETP	16q21	14328	68805	54477
rs1047699	PROL4	12p13	2424	93715	91291
rs1948017	GRID2	4q22	206	90969	90763
rs1498608	PDE4D	5q12	1599	82591	80992
rs869975	GPX3/TNIP1	5q23	231	86539	86308

Additional Polymorphic Variants Associated with Low BMD

[0040] Also provided is a method for identifying polymorphic variants proximal to an incident, founder polymorphic variant associated with low BMD. Thus, featured herein are methods for

identifying a polymorphic variation associated with low BMD that is proximal to an incident polymorphic variation associated with low BMD, which comprises identifying a polymorphic variant proximal to the incident polymorphic variant associated with low BMD, where the incident polymorphic variant is in a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence. The nucleotide sequence often comprises a polynucleotide sequence selected from the group consisting of (a) a polynucleotide sequence of SEQ ID NO's:1-5; (b) a polynucleotide sequence that encodes a polypeptide having an amino acid sequence encoded by a polynucleotide sequence of SEQ ID NO's:1-5; and (c) a polynucleotide sequence that encodes a polypeptide having an amino acid sequence that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-5 or a polynucleotide sequence 90% or more identical to the polynucleotide sequence of SEQ ID NO's:1-5. The presence or absence of an association of the proximal polymorphic variant with low BMD then is determined using a known association method, such as a method described in the Examples hereafter. In an embodiment, the incident polymorphic variant is a polymorphic variant associated with low BMD described herein. In another embodiment, the proximal polymorphic variant identified sometimes is a publicly disclosed polymorphic variant, which for example, sometimes is published in a publicly available database. In other embodiments, the polymorphic variant identified is not publicly disclosed and is discovered using a known method, including, but not limited to, sequencing a region surrounding the incident polymorphic variant in a group of nucleic samples. Thus, multiple polymorphic variants proximal to an incident polymorphic variant are associated with low BMD using this method.

[0041] The proximal polymorphic variant often is identified in a region surrounding the incident polymorphic variant. In certain embodiments, this surrounding region is about 50 kb flanking the first polymorphic variant (*e.g.* about 50 kb 5' of the first polymorphic variant and about 50 kb 3' of the first polymorphic variant), and the region sometimes is composed of shorter flanking sequences, such as flanking sequences of about 40 kb, about 30 kb, about 25 kb, about 20 kb, about 15 kb, about 10 kb, about 7 kb, about 5 kb, or about 2 kb 5' and 3' of the incident polymorphic variant. In other embodiments, the region is composed of longer flanking sequences, such as flanking sequences of about 55 kb, about 60 kb, about 65 kb, about 70 kb, about 75 kb, about 80 kb, about 85 kb, about 90 kb, about 95 kb, or about 100 kb 5' and 3' of the incident polymorphic variant.

[0042] In certain embodiments, polymorphic variants associated with low BMD are identified iteratively. For example, a first proximal polymorphic variant is associated with low BMD using the methods described above and then another polymorphic variant proximal to the first proximal polymorphic variant is identified (*e.g.*, publicly disclosed or discovered) and the presence or absence of an association of one or more other polymorphic variants proximal to the first proximal polymorphic variant with low BMD is determined.

[0043] The methods described herein are useful for identifying or discovering additional polymorphic variants that may be used to further characterize a gene, region or loci associated with a

condition, a disease (*e.g.*, osteoporosis), or a disorder. For example, allelotyping or genotyping data from the additional polymorphic variants may be used to identify a functional mutation or a region of linkage disequilibrium. In certain embodiments, polymorphic variants identified or discovered within a region comprising the first polymorphic variant associated with low BMD are genotyped using the genetic methods and sample selection techniques described herein, and it can be determined whether those polymorphic variants are in linkage disequilibrium with the first polymorphic variant. The size of the region in linkage disequilibrium with the first polymorphic variant also can be assessed using these genotyping methods. Thus, provided herein are methods for determining whether a polymorphic variant is in linkage disequilibrium with a first polymorphic variant associated with low BMD, and such information can be used in prognosis/diagnosis methods described herein.

Isolated Nucleic Acids

[0044] Featured herein are isolated *CETP, PROLA, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid variants depicted in SEQ ID NO's:1-10, and substantially identical nucleic acids thereof. A nucleic acid variant may be represented on one or both strands in a double-stranded nucleic acid or on one chromosomal complement (heterozygous) or both chromosomal complements (homozygous)).

[0045] As used herein, the term "nucleic acid" includes DNA molecules (*e.g.*, a complementary DNA (cDNA) and genomic DNA (gDNA)) and RNA molecules (*e.g.*, mRNA, rRNA, siRNA and tRNA) and analogs of DNA or RNA, for example, by use of nucleotide analogs. The nucleic acid molecule can be single-stranded and it is often double-stranded. The term "isolated or purified nucleic acid" refers to nucleic acids that are separated from other nucleic acids present in the natural source of the nucleic acid. For example, with regard to genomic DNA, the term "isolated" includes nucleic acids which are separated from the chromosome with which the genomic DNA is naturally associated. An "isolated" nucleic acid is often free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. As used herein, the term "gene" refers to a nucleotide sequence that encodes a polypeptide.

[0046] The nucleic acid often comprises a part of or all of a nucleotide sequence in SEQ ID NO's:1-5, or a substantially identical sequence thereof. Such a nucleotide sequence sometimes is a 5' and/or 3' sequence flanking a polymorphic variant described above that is 5-1000 nucleotides in length, or in some embodiments 5-500, 5-100, 5-75, 5-50, 5-45, 5-40, 5-35, 5-30, 5-25 or 5-20 nucleotides in

length. Other embodiments are directed to methods of identifying a polymorphic variation at one or more positions in a nucleic acid (*e.g.*, genotyping at one or more positions in the nucleic acid), such as at a position corresponding to rs1801706 in the CETP gene, rs1047699 in the PROL4 gene, rs1948017 in the GRID2 gene, rs1498608 in the PDE4D gene, or rs869975 in the GPX3 gene.

[0047] Also included herein are nucleic acid fragments. These fragments often are a nucleotide sequence identical to a nucleotide sequence of SEQ ID NO's:1-10, a nucleotide sequence substantially identical to a nucleotide sequence of SEQ ID NO's:1-10, or a nucleotide sequence that is complementary to the foregoing. The nucleic acid fragment may be identical, substantially identical or homologous to a nucleotide sequence in an exon or an intron in a nucleotide sequence of SEQ ID NO's:1-5, and may encode a domain or part of a domain of a polypeptide. Sometimes, the fragment will comprises one or more of the polymorphic variations described herein as being associated with low BMD. The nucleic acid fragment is often 50, 100, or 200 or fewer base pairs in length, and is sometimes about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 2000, 3000, 4000, 5000, 10000, 15000, or 20000 base pairs in length. A nucleic acid fragment that is complementary to a nucleotide sequence identical or substantially identical to a nucleotide sequence in SEQ ID NO's:1-5 and hybridizes to such a nucleotide sequence under stringent conditions is often referred to as a "probe." Nucleic acid fragments often include one or more polymorphic sites, or sometimes have an end that is adjacent to a polymorphic site as described hereafter. *CETP* nucleic acid fragments sometimes encode the mature protein from positions 182 to 1609 of the mRNA sequence (SEQ ID NO: 6), for example.

[0048] An example of a nucleic acid fragment is an oligonucleotide. As used herein, the term "oligonucleotide" refers to a nucleic acid comprising about 8 to about 50 covalently linked nucleotides, often comprising from about 8 to about 35 nucleotides, and more often from about 10 to about 25 nucleotides. The backbone and nucleotides within an oligonucleotide may be the same as those of naturally occurring nucleic acids, or analogs or derivatives of naturally occurring nucleic acids, provided that oligonucleotides having such analogs or derivatives retain the ability to hybridize specifically to a nucleic acid comprising a targeted polymorphism. Oligonucleotides described herein may be used as hybridization probes or as components of prognostic or diagnostic assays, for example, as described herein.

[0049] Oligonucleotides are typically synthesized using standard methods and equipment, such as the ABI™3900 High Throughput DNA Synthesizer and the EXPEDITE™ 8909 Nucleic Acid Synthesizer, both of which are available from Applied Biosystems (Foster City, CA). Analogs and derivatives are exemplified in U.S. Pat. Nos. 4,469,863; 5,536,821; 5,541,306; 5,637,683; 5,637,684; 5,700,922; 5,717,083; 5,719,262; 5,739,308; 5,773,601; 5,886,165; 5,929,226; 5,977,296; 6,140,482; WO 00/56746; WO 01/14398, and related publications. Methods for synthesizing oligonucleotides comprising such analogs or derivatives are disclosed, for example, in the patent publications cited above and in U.S. Pat. Nos. 5,614,622; 5,739,314; 5,955,599; 5,962,674; 6,117,992; in WO 00/75372; and in related publications.

[0050] Oligonucleotides may also be linked to a second moiety. The second moiety may be an additional nucleotide sequence such as a tail sequence (*e.g.*, a polyadenosine tail), an adapter sequence (*e.g.*, phage M13 universal tail sequence), and others. Alternatively, the second moiety may be a non-nucleotide moiety such as a moiety which facilitates linkage to a solid support or a label to facilitate detection of the oligonucleotide. Such labels include, without limitation, a radioactive label, a fluorescent label, a chemiluminescent label, a paramagnetic label, and the like. The second moiety may be attached to any position of the oligonucleotide, provided the oligonucleotide can hybridize to the nucleic acid comprising the polymorphism.

Uses of Nucleic Acid Sequence

[0051] Nucleic acid coding sequences (*e.g.*, SEQ ID NO: 7-12) may be used for diagnostic purposes for detection and control of polypeptide expression. Also, included herein are oligonucleotide sequences such as antisense RNA, small-interfering RNA (siRNA) and DNA molecules and ribozymes that function to inhibit translation of a polypeptide. Antisense techniques and RNA interference techniques are known in the art and are described herein.

[0052] Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, hammerhead motif ribozyme molecules may be engineered that specifically and efficiently catalyze endonucleolytic cleavage of RNA sequences corresponding to or complementary to *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequences. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between fifteen (15) and twenty (20) ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

[0053] Antisense RNA and DNA molecules, siRNA and ribozymes may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

[0054] DNA encoding a polypeptide also may have a number of uses for the diagnosis of diseases, including low BMD, resulting from aberrant expression of a target gene described herein. For example, the nucleic acid sequence may be used in hybridization assays of biopsies or autopsies to diagnose abnormalities of expression or function (*e.g.*, Southern or Northern blot analysis, in situ hybridization assays).

[0055] In addition, the expression of a polypeptide during embryonic development may also be determined using nucleic acid encoding the polypeptide. As addressed, *infra*, production of functionally impaired polypeptide is the cause of various disease states, such as osteoporosis. *In situ* hybridizations using polypeptide as a probe may be employed to predict problems related to low BMD. Further, as indicated, *infra*, administration of human active polypeptide, recombinantly produced as described herein, may be used to treat disease states related to functionally impaired polypeptide. Alternatively, gene therapy approaches may be employed to remedy deficiencies of functional polypeptide or to replace or compete with dysfunctional polypeptide.

Expression Vectors, Host Cells, and Genetically Engineered Cells

[0056] Provided herein are nucleic acid vectors, often expression vectors, which contain a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence or a substantially identical sequence thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid, or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors may include replication defective retroviruses, adenoviruses and adeno-associated viruses for example.

[0057] A vector can include a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence in a form suitable for expression of an encoded target polypeptide or target nucleic acid in a host cell. A "target polypeptide" is a polypeptide encoded by a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence or a substantially identical nucleotide sequence thereof. The recombinant expression vector typically includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, and the like. Expression vectors can be introduced into host cells to produce target polypeptides, including fusion polypeptides.

[0058] Recombinant expression vectors can be designed for expression of target polypeptides in prokaryotic or eukaryotic cells. For example, target polypeptides can be expressed in *E. coli*, insect cells (*e.g.*, using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology 185*,

Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[0059] Expression of polypeptides in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant polypeptide; 2) to increase the solubility of the recombinant polypeptide; and 3) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion polypeptide. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith & Johnson, *Gene 67:* 31-40 (1988)), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding polypeptide, or polypeptide A, respectively, to the target recombinant polypeptide.

[0060] Purified fusion polypeptides can be used in screening assays and to generate antibodies specific for target polypeptides. In a therapeutic embodiment, fusion polypeptide expressed in a retroviral expression vector is used to infect bone marrow cells that are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six (6) weeks).

[0061] Expressing the polypeptide in host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide is often used to maximize recombinant polypeptide expression (Gottesman, S., *Gene Expression Technology: Methods in Enzymology, Academic Press, San Diego, California 185:* 119-128 (1990)). Another strategy is to alter the nucleotide sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al., Nucleic Acids Res. 20:* 2111-2118 (1992)). Such alteration of nucleotide sequences can be carried out by standard DNA synthesis techniques.

[0062] When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. Recombinant mammalian expression vectors are often capable of directing expression of the nucleic acid in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include an albumin promoter (liver-specific; Pinkert *et al., Genes Dev. 1:* 268-277 (1987)), lymphoid-specific promoters (Calame & Eaton, *Adv. Immunol. 43:* 235-275 (1988)), promoters of T cell receptors (Winoto & Baltimore, *EMBO J. 8:* 729-733 (1989)) promoters of

immunoglobulins (Banerji *et al., Cell 33:* 729-740 (1983); Queen & Baltimore, *Cell 33:* 741-948 (1983)), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne & Ruddle, *Proc. Natl. Acad. Sci. USA 86:* 5473-5477 (1989)), pancreas-specific promoters (Edlund *et al., Science 230:* 912-916 (1985)), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are sometimes utilized, for example, the murine hox promoters (Kessel & Gruss, *Science 249:* 374-379 (1990)) and the α-fetopolypeptide promoter (Campes & Tilghman, *Genes Dev. 3:* 537-546 (1989)).

[0063] A *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid may also be cloned into an expression vector in an antisense orientation. Regulatory sequences (*e.g.*, viral promoters and/or enhancers) operatively linked to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid cloned in the antisense orientation can be chosen for directing constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. Antisense expression vectors can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) (1986).

[0064] Also provided herein are host cells that include a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence within a recombinant expression vector or a fragment of such a nucleotide sequence which facilitate homologous recombination into a specific site of the host cell genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but rather also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell can be any prokaryotic or eukaryotic cell. For example, a target polypeptide can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

[0065] Vectors can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, transduction/infection, DEAE-dextran-mediated transfection, lipofection, or electroporation.

[0066] A host cell provided herein can be used to produce (*i.e.*, express) a target polypeptide or a substantially identical polypeptide thereof. Accordingly, further provided are methods for producing a target polypeptide using host cells described herein. In one embodiment, the method includes culturing host cells into which a recombinant expression vector encoding a target polypeptide has been introduced in a suitable medium such that a target polypeptide is produced. In another embodiment, the method further includes isolating a target polypeptide from the medium or the host cell.

[0067] Also provided are cells or purified preparations of cells which include a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* transgene, or which otherwise misexpress target polypeptide. Cell preparations can consist of human or non-human cells, *e.g.*, rodent cells, *e.g.*, mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* transgene (*e.g.*, a heterologous form of a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* gene, such as a human gene expressed in non-human cells). The transgene can be misexpressed, *e.g.*, overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous target polypeptide (*e.g.*, expression of a gene is disrupted, also known as a knockout). Such cells can serve as a model for studying disorders which are related to mutated or mis-expressed alleles or for use in drug screening. Also provided are human cells (*e.g.*, a hematopoietic stem cells) transformed with a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid.

[0068] Also provided are cells or a purified preparation thereof (*e.g.*, human cells) in which an endogenous *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid is under the control of a regulatory sequence that does not normally control the expression of the endogenous gene corresponding to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence. The expression characteristics of an endogenous gene within a cell (*e.g.*, a cell line or microorganism) can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the corresponding endogenous gene. For example, an endogenous corresponding gene (*e.g.*, a gene which is "transcriptionally silent," not normally expressed, or expressed only at very low levels) may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, *e.g.*, Chappel, US 5,272,071; WO 91/06667, published on May 16, 1991.

Transgenic Animals

[0069] Non-human transgenic animals that express a heterologous target polypeptide (*e.g.*, expressed from a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid or substantially identical sequence thereof) can be generated. Such animals are useful for studying the function and/or activity of a target polypeptide and for identifying and/or evaluating modulators of the activity of *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acids and encoded polypeptides. As used herein, a "transgenic animal" is a non-human animal such as a mammal (*e.g.*, a non-human primate such as chimpanzee, baboon, or macaque; an ungulate such as an equine, bovine, or caprine; or a rodent such as a rat, a mouse, or an Israeli sand rat), a bird (*e.g.*, a chicken or a turkey), an amphibian (*e.g.*, a frog, salamander, or newt), or an insect (*e.g.*, *Drosophila melanogaster*), in which one or more of the cells of the animal includes a transgene. A transgene is exogenous DNA or a rearrangement (*e.g.*, a deletion of endogenous chromosomal DNA) that is often integrated into or occurs in the genome of

cells in a transgenic animal. A transgene can direct expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, and other transgenes can reduce expression (*e.g.*, a knockout). Thus, a transgenic animal can be one in which an endogenous nucleic acid homologous to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal (*e.g.*, an embryonic cell of the animal) prior to development of the animal.

[0070] Intronic sequences and polyadenylation signals can also be included in the transgene to increase expression efficiency of the transgene. One or more tissue-specific regulatory sequences can be operably linked to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence to direct expression of an encoded polypeptide to particular cells. A transgenic founder animal can be identified based upon the presence of a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence in its genome and/or expression of encoded mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence can further be bred to other transgenic animals carrying other transgenes.

[0071] Target polypeptides can be expressed in transgenic animals or plants by introducing, for example, a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid into the genome of an animal that encodes the target polypeptide. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, *e.g.*, a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Also included is a population of cells from a transgenic animal.

Target Polypeptides

[0072] Also featured herein are isolated target polypeptides, which are encoded by a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence (*e.g.*, SEQ ID NO's:1-10) or a substantially identical nucleotide sequence thereof, such as the polypeptides having amino acid sequences in SEQ ID NO's:11-15. The term "polypeptide" as used herein includes proteins and peptides. An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means preparation of a target polypeptide having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-target polypeptide (also referred to herein as a "contaminating protein"), or of chemical precursors or non-target chemicals. When the target polypeptide or a biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, specifically, where culture medium represents less than about 20%, sometimes less than about 10%, and often less than about 5% of the volume of the polypeptide preparation. Isolated or purified target polypeptide preparations are sometimes 0.01

milligrams or more or 0.1 milligrams or more, and often 1.0 milligrams or more and 10 milligrams or more in dry weight.

[0073] Further included herein are target polypeptide fragments. The polypeptide fragment may be a domain or part of a domain of a target polypeptide. In addition, the polypeptide fragment may be a full-length polypeptide or a mature polypeptide (*i.e.*, the polypeptide minus the signal peptide). For example, a fragment sometimes is a *CTEP* mature protein that corresponds to amino acid positions 18-493 of SEQ ID NO:11. The polypeptide fragment may have increased, decreased or unexpected biological activity. The polypeptide fragment is often 50 or fewer, 100 or fewer, or 200 or fewer amino acids in length, and is sometimes 300, 400, 500, 600, 700, or 900 or fewer amino acids in length.

[0074] Substantially identical target polypeptides may depart from the amino acid sequences of target polypeptides in different manners. For example, conservative amino acid modifications may be introduced at one or more positions in the amino acid sequences of target polypeptides. A "conservative amino acid substitution" is one in which the amino acid is replaced by another amino acid having a similar structure and/or chemical function. Families of amino acid residues having similar structures and functions are well known. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Also, essential and non-essential amino acids may be replaced. A "non-essential" amino acid is one that can be altered without abolishing or substantially altering the biological function of a target polypeptide, whereas altering an "essential" amino acid abolishes or substantially alters the biological function of a target polypeptide. Amino acids that are conserved among target polypeptides are typically essential amino acids.

[0075] Also, target polypeptides may exist as chimeric or fusion polypeptides. As used herein, a target "chimeric polypeptide" or target "fusion polypeptide" includes a target polypeptide linked to a non-target polypeptide. A "non-target polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a polypeptide which is not substantially identical to the target polypeptide, which includes, for example, a polypeptide that is different from the target polypeptide and derived from the same or a different organism. The target polypeptide in the fusion polypeptide can correspond to an entire or nearly entire target polypeptide or a fragment thereof. The non-target polypeptide can be fused to the N-terminus or C-terminus of the target polypeptide.

[0076] Fusion polypeptides can include a moiety having high affinity for a ligand. For example, the fusion polypeptide can be a GST-target fusion polypeptide in which the target sequences are fused to the C-terminus of the GST sequences, or a polyhistidine-target fusion polypeptide in which the target polypeptide is fused at the N- or C-terminus to a string of histidine residues. Such fusion polypeptides can facilitate purification of recombinant target polypeptide. Expression vectors are commercially

available that already encode a fusion moiety (*e.g.*, a GST polypeptide), and a nucleotide sequence in SEQ ID NO's:1-10, or a substantially identical nucleotide sequence thereof, can be cloned into an expression vector such that the fusion moiety is linked in-frame to the target polypeptide. Further, the fusion polypeptide can be a target polypeptide containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression, secretion, cellular internalization, and cellular localization of a target polypeptide can be increased through use of a heterologous signal sequence. Fusion polypeptides can also include all or a part of a serum polypeptide (*e.g.*, an IgG constant region or human serum albumin).

[0077] Target polypeptides can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. Administration of these target polypeptides can be used to affect the bioavailability of a substrate of the target polypeptide and may effectively increase target polypeptide biological activity in a cell. Target fusion polypeptides may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a target polypeptide; (ii) mis-regulation of the gene encoding the target polypeptide; and (iii) aberrant post-translational modification of a target polypeptide. Also, target polypeptides can be used as immunogens to produce anti-target antibodies in a subject, to purify target polypeptide ligands or binding partners, and in screening assays to identify molecules which inhibit or enhance the interaction of a target polypeptide with a substrate.

[0078] In addition, polypeptides can be chemically synthesized using techniques known in the art (See, *e.g.*, Creighton, 1983 Proteins. New York, N.Y.: W. H. Freeman and Company; and Hunkapiller *et al.*, (1984) Nature July 12 -18;310(5973):105-11). For example, a relative short fragment can be synthesized by use of a peptide synthesizer. Furthermore, if desired, non-classical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the fragment sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoroamino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0079] Polypeptides and polypeptide fragments sometimes are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH4; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; and the like. Additional post-translational modifications include, for

example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptide fragments may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the polypeptide.

[0080] Also provided are chemically modified derivatives of polypeptides that can provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (*see e.g.*, U.S. Pat. No: 4,179,337. The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0081] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (*e.g.*, the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

[0082] The polymers should be attached to the polypeptide with consideration of effects on functional or antigenic domains of the polypeptide. There are a number of attachment methods available to those skilled in the art (*e.g.*, EP 0 401 384 (coupling PEG to G-CSF) and Malik *et al.* (1992) Exp Hematol. September;20(8):1028-35 (pegylation of GM-CSF using tresyl chloride)). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. For therapeutic purposes, the attachment sometimes is at an amino group, such as attachment at the N-terminus or lysine group.

[0083] Proteins can be chemically modified at the N-terminus. Using polyethylene glycol as an illustration of such a composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, and the like), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-

terminally pegylated preparation (*i.e.*, separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus may be accomplished by reductive alkylation, which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

Substantially Identical Nucleic Acids and Polypeptides

[0084] Nucleotide sequences and polypeptide sequences that are substantially identical to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence and the target polypeptide sequences encoded by those nucleotide sequences, respectively, are included herein. The term "substantially identical" as used herein refers to two or more nucleic acids or polypeptides sharing one or more identical nucleotide sequences or polypeptide sequences, respectively. Included are nucleotide sequences or polypeptide sequences that are 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more (each often within a 1%, 2%, 3% or 4% variability) identical to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence or the encoded target polypeptide amino acid sequences. One test for determining whether two nucleic acids are substantially identical is to determine the percent of identical nucleotide sequences or polypeptide sequences shared between the nucleic acids or polypeptides.

[0085] Calculations of sequence identity are often performed as follows. Sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The length of a reference sequence aligned for comparison purposes is sometimes 30% or more, 40% or more, 50% or more, often 60% or more, and more often 70% or more, 80% or more, 90% or more, or 100% of the length of the reference sequence. The nucleotides or amino acids at corresponding nucleotide or polypeptide positions, respectively, are then compared among the two sequences. When a position in the first sequence is occupied by the same nucleotide or amino acid as the corresponding position in the second sequence, the nucleotides or amino acids are deemed to be identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, introduced for optimal alignment of the two sequences.

[0086] Comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. Percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of Meyers & Miller, *CABIOS 4:* 11-17 (1989), which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight

residue table, a gap length penalty of 12 and a gap penalty of 4. Also, percent identity between two amino acid sequences can be determined using the Needleman & Wunsch, *J. Mol. Biol. 48:* 444-453 (1970) algorithm which has been incorporated into the GAP program in the GCG software package (available at the http address www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. Percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http address www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A set of parameters often used is a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0087] Another manner for determining if two nucleic acids are substantially identical is to assess whether a polynucleotide homologous to one nucleic acid will hybridize to the other nucleic acid under stringent conditions. As use herein, the term "stringent conditions" refers to conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y., 6.3.1-6.3.6 (1989). Aqueous and non-aqueous methods are described in that reference and either can be used. An example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Often, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. More often, stringency conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C.

[0088] An example of a substantially identical nucleotide sequence to a nucleotide sequence in SEQ ID NO's:1-10 is one that has a different nucleotide sequence but still encodes the same polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO's:1-10. Another example is a nucleotide sequence that encodes a polypeptide having a polypeptide sequence that is more than 70% or more identical to, sometimes more than 75% or more, 80% or more, or 85% or more identical to, and often more than 90% or more and 95% or more identical to a polypeptide sequence encoded by a nucleotide sequence in SEQ ID NO's:1-10. As used herein, "SEQ ID NO's:1-10" typically refers to one or more sequences in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, and/or 10. Many of the embodiments described herein are applicable to (a) a nucleotide sequence of SEQ ID NO's:1-10; (b) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-10; (c) a nucleotide sequence which encodes a polypeptide that is 90% or

more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-10, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO's:1-10; (d) a fragment of a nucleotide sequence of (a), (b), or (c); and/or a nucleotide sequence complementary to the nucleotide sequences of (a), (b), (c) and/or (d), where nucleotide sequences of (b) and (c), fragments of (b) and (c) and nucleotide sequences complementary to (b) and (c) are examples of substantially identical nucleotide sequences. Examples of substantially identical nucleotide sequences include nucleotide sequences from subjects that differ by naturally occurring genetic variance, which sometimes is referred to as background genetic variance (*e.g.*, nucleotide sequences differing by natural genetic variance sometimes are 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to one another).

[0089] Nucleotide sequences in SEQ ID NO's:1-10 and amino acid sequences of encoded polypeptides can be used as "query sequences" to perform a search against public databases to identify other family members or related sequences, for example. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al., J. Mol. Biol. 215:* 403-10 (1990). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleotide sequences in SEQ ID NO's:1-10. BLAST polypeptide searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to polypeptides encoded by the nucleotide sequences of SEQ ID NO's:1-10. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al., Nucleic Acids Res. 25(17):* 3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (*see* the http address www.ncbi.nlm.nih.gov).

[0090] A nucleic acid that is substantially identical to a nucleotide sequence in SEQ ID NO's:1-10 may include polymorphic sites at positions equivalent to those described herein when the sequences are aligned. For example, using the alignment procedures described herein, SNPs in a sequence substantially identical to a sequence in SEQ ID NO's:1-10 can be identified at nucleotide positions that match with or correspond to (*i.e.*, align) nucleotides at SNP positions in each nucleotide sequence in SEQ ID NO's:1-10. Also, where a polymorphic variation results in an insertion or deletion, insertion or deletion of a nucleotide sequence from a reference sequence can change the relative positions of other polymorphic sites in the nucleotide sequence.

[0091] Substantially identical nucleotide and polypeptide sequences include those that are naturally occurring, such as allelic variants (same locus), splice variants, homologs (different locus), and orthologs (different organism) or can be non-naturally occurring. Non-naturally occurring variants can be generated by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

Orthologs, homologs, allelic variants, and splice variants can be identified using methods known in the art. These variants normally comprise a nucleotide sequence encoding a polypeptide that is 50% or more, about 55% or more, often about 70-75% or more or about 80-85% or more, and sometimes about 90-95% or more identical to the amino acid sequences of target polypeptides or a fragment thereof. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions to a nucleotide sequence in SEQ ID NO's:1-10 or a fragment of this sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of a nucleotide sequence in SEQ ID NO's:1-10 can further be identified by mapping the sequence to the same chromosome or locus as the nucleotide sequence in SEQ ID NO's:1-10.

[0092] Also, substantially identical nucleotide sequences may include codons that are altered with respect to the naturally occurring sequence for enhancing expression of a target polypeptide in a particular expression system. For example, the nucleic acid can be one in which one or more codons are altered, and often 10% or more or 20% or more of the codons are altered for optimized expression in bacteria (*e.g.*, *E. coli*.), yeast (*e.g.*, *S. cervesiae*), human (*e.g.*, 293 cells), insect, or rodent (*e.g.*, hamster) cells.

Methods for Identifying Subjects at Risk of Osteoporosis and Risk of Osteoporosis in a Subject

[0093] Methods for prognosing and diagnosing low BMD and its related disorders (*e.g.*, osteoporosis) are included herein. These methods include detecting the presence or absence of one or more polymorphic variations in a nucleotide sequence associated with low BMD, such as variants in or around the loci set forth herein, or a substantially identical sequence thereof, in a sample from a subject, where the presence of a polymorphic variant described herein is indicative of a risk of low BMD or one or more low BMD related disorders (*e.g.*, osteoporosis). Determining a risk of osteoporosis refers to determining whether an individual is at an increased risk of osteoporosis (*e.g.*, intermediate risk or higher risk).

[0094] Thus, featured herein is a method for identifying a subject who is at risk of osteoporosis, which comprises detecting low BMD-associated aberration in a nucleic acid sample from the subject. An embodiment is a method for detecting a risk of osteoporosis in a subject, which comprises detecting the presence or absence of a polymorphic variation associated with low BMD at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject, where the nucleotide sequence comprises a polynucleotide sequence selected from the group consisting of: (a) a nucleotide sequence of SEQ ID NO's:1-10; (b) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-10; (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-10, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO's:1-10; and (d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising the polymorphic site; whereby the presence of the polymorphic variation is indicative of a

predisposition to osteoporosis in the subject. In certain embodiments, polymorphic variants at the positions described herein are detected for determining a risk of osteoporosis, and polymorphic variants at positions in linkage disequilibrium with these positions are detected for determining a risk of osteoporosis. As used herein, "SEQ ID NO's:1-10" refers to individual sequences in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12.

[0095] Results from prognostic tests may be combined with other test results to diagnose osteoporosis. For example, prognostic results may be gathered, an initial diagnostic test may be ordered based on a determined predisposition to low BMD, and the results of the analysis may be utilized to diagnose osteoporosis. Also osteoporosis diagnostic methods can be developed from studies used to generate prognostic/diagnostic methods in which populations are stratified into subpopulations having different progressions of osteoporosis. In another embodiment, prognostic results may be gathered; a patient's risk factors for developing osteoporosis analyzed (*e.g.*, age, race, family history, age of menopause); and an initial diagnostic test may be ordered based on a determined predisposition to low BMD.

Risk factors believed to be associated with low BMD include personal history of fracture after age 50; current low bone mass; history of fracture in a 1° relative; being female; being thin and/or having a small frame; low body weight; advanced age; a family history of osteoporosis; estrogen deficiency, especially as a result of menopause which is early or surgically induced; abnormal absence of menstrual periods (amenorrhea); anorexia nervosa; low lifetime calcium intake; use of certain medications, such as corticosteroids and anticonvulsants; low testosterone levels in men; an inactive lifestyle; current cigarette smoking; excessive use of alcohol; being Caucasian or Asian, although African Americans and Hispanic Americans are at significant risk as well. (See National Osteoporosis Foundation; http://www.nof.org/osteoporosis/stats.htm).

In an alternative embodiment, the results from predisposition analyses described herein may be combined with other test results indicative of osteoporosis, which were previously, concurrently, or subsequently gathered with respect to the predisposition testing. In these embodiments, the combination of the prognostic test results with other test results can be probative of osteoporosis, and the combination can be utilized as an osteoporosis diagnostic. The results of any test indicative of osteoporosis known in the art may be combined with the methods described herein. Examples of such tests are bone density tests that measure bone density in various sites of the body. Such methods include the measurement of the radiodensity of skeletal radiographs, quantitative computerized tomography, single energy photon absorptiometry, and dual-energy photon absorptiometry. Diagnostic techniques among those useful herein are described in W. A. Peck et al., Physician's Resource Manual on Osteoporosis (1987), published by the National Osteoporosis Foundation (incorporated by reference herein).

[0096] Risk of low BMD sometimes is expressed as a probability, such as an odds ratio, percentage, or risk factor. The risk sometimes is expressed as a relative risk with respect to a

population average risk of low BMD, and sometimes is expressed as a relative risk with respect to the lowest risk group. Such relative risk assessments often are based upon penetrance values determined by statistical methods and are particularly useful to clinicians and insurance companies for assessing risk of osteoporosis (*e.g.*, a clinician can target appropriate detection, prevention and therapeutic regimens to a patient after determining the patient's risk of osteoporosis, and an insurance company can fine tune actuarial tables based upon population genotype assessments of osteoporosis risk). Risk of osteoporosis sometimes is expressed as an odds ratio, which is the odds of a particular person having a genotype has or will develop osteoporosis with respect to another genotype group (*e.g.*, the most disease protective genotype or population average). The risk often is based upon the presence or absence of one or more polymorphic variants described herein, and also may be based in part upon phenotypic traits of the individual being tested. In an embodiment, two or more polymorphic variations are detected in a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* locus. In certain embodiments, 3 or more, or 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more polymorphic variants are detected in the sample. Methods for calculating risk based upon patient data are well known (*see, e.g.*, Agresti, *Categorical Data Analysis*, 2nd Ed. 2002. Wiley). Allelotyping and genotyping analyses may be carried out in populations other than those exemplified herein to enhance the predictive power of the prognostic method.

[0097] The nucleic acid sample typically is isolated from a biological sample obtained from a subject. For example, nucleic acid can be isolated from blood, saliva, sputum, urine, cell scrapings, and biopsy tissue. The nucleic acid sample can be isolated from a biological sample using standard techniques, such as the technique described in Example 2. As used herein, the term "subject" refers primarily to humans but also refers to other mammals such as dogs, cats, and ungulates (*e.g.*, cattle, sheep, and swine). Subjects also include avians (*e.g.*, chickens and turkeys), reptiles, and fish (*e.g.*, salmon), as embodiments described herein can be adapted to nucleic acid samples isolated from any of these organisms. The nucleic acid sample may be isolated from the subject and then directly utilized in a method for determining the presence of a polymorphic variant, or alternatively, the sample may be isolated and then stored (*e.g.*, frozen) for a period of time before being subjected to analysis.

[0098] The presence or absence of a polymorphic variant is determined using one or both chromosomal complements represented in the nucleic acid sample. Determining the presence or absence of a polymorphic variant in both chromosomal complements represented in a nucleic acid sample from a subject having a copy of each chromosome is useful for determining the zygosity of an individual for the polymorphic variant (*i.e.*, whether the individual is homozygous or heterozygous for the polymorphic variant). Any oligonucleotide-based diagnostic may be utilized to determine whether a sample includes the presence or absence of a polymorphic variant in a sample. For example, primer extension methods, ligase sequence determination methods (*e.g.*, U.S. Pat. Nos. 5,679,524 and 5,952,174, and WO 01/27326), mismatch sequence determination methods (*e.g.*, U.S. Pat. Nos. 5,851,770; 5,958,692; 6,110,684; and 6,183,958), microarray sequence determination methods,

restriction fragment length polymorphism (RFLP), single strand conformation polymorphism detection (SSCP) (*e.g.*, U.S. Pat. Nos. 5,891,625 and 6,013,499), PCR-based assays (*e.g.*, TAQMAN® PCR System (Applied Biosystems)), and nucleotide sequencing methods may be used.

[0099] Oligonucleotide extension methods typically involve providing a pair of oligonucleotide primers in a polymerase chain reaction (PCR) or in other nucleic acid amplification methods for the purpose of amplifying a region from the nucleic acid sample that comprises the polymorphic variation. One oligonucleotide primer is complementary to a region 3' of the polymorphism and the other is complementary to a region 5' of the polymorphism. A PCR primer pair may be used in methods disclosed in U.S. Pat. Nos. 4,683,195; 4,683,202, 4,965,188; 5,656,493; 5,998,143; 6,140,054; WO 01/27327; and WO 01/27329 for example. PCR primer pairs may also be used in any commercially available machines that perform PCR, such as any of the GENEAMP® Systems available from Applied Biosystems. Also, those of ordinary skill in the art will be able to design oligonucleotide primers based upon a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence using knowledge available in the art.

[0100] Also provided is an extension oligonucleotide that hybridizes to the amplified fragment adjacent to the polymorphic variation. As used herein, the term "adjacent" refers to the 3' end of the extension oligonucleotide being often 1 nucleotide from the 5' end of the polymorphic site, and sometimes 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides from the 5' end of the polymorphic site, in the nucleic acid when the extension oligonucleotide is hybridized to the nucleic acid. The extension oligonucleotide then is extended by one or more nucleotides, and the number and/or type of nucleotides that are added to the extension oligonucleotide determine whether the polymorphic variant is present. Oligonucleotide extension methods are disclosed, for example, in U.S. Pat. Nos. 4,656,127; 4,851,331; 5,679,524; 5,834,189; 5,876,934; 5,908,755; 5,912,118; 5,976,802; 5,981,186; 6,004,744; 6,013,431; 6,017,702; 6,046,005; 6,087,095; 6,210,891; and WO 01/20039. Oligonucleotide extension methods using mass spectrometry are described, for example, in U.S. Pat. Nos. 5,547,835; 5,605,798; 5,691,141; 5,849,542; 5,869,242; 5,928,906; 6,043,031; and 6,194,144, and a method often utilized is described herein in Example 2.

[0101] A microarray can be utilized for determining whether a polymorphic variant is present or absent in a nucleic acid sample. A microarray may include any oligonucleotides described herein, and methods for making and using oligonucleotide microarrays suitable for diagnostic use are disclosed in U.S. Pat. Nos. 5,492,806; 5,525,464; 5,589,330; 5,695,940; 5,849,483; 6,018,041; 6,045,996; 6,136,541; 6,142,681; 6,156,501; 6,197,506; 6,223,127; 6,225,625; 6,229,911; 6,239,273; WO 00/52625; WO 01/25485; and WO 01/29259. The microarray typically comprises a solid support and the oligonucleotides may be linked to this solid support by covalent bonds or by non-covalent interactions. The oligonucleotides may also be linked to the solid support directly or by a spacer molecule. A microarray may comprise one or more oligonucleotides complementary to a polymorphic site set forth herein.

[0102] A kit also may be utilized for determining whether a polymorphic variant is present or absent in a nucleic acid sample. A kit often comprises one or more pairs of oligonucleotide primers useful for amplifying a fragment of a nucleotide sequence of SEQ ID NO's:1-10 or a substantially identical sequence thereof, where the fragment includes a polymorphic site. The kit sometimes comprises a polymerizing agent, for example, a thermostable nucleic acid polymerase such as one disclosed in U.S. Pat. Nos. 4,889,818 or 6,077,664. Also, the kit often comprises an elongation oligonucleotide that hybridizes to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence in a nucleic acid sample adjacent to the polymorphic site. Where the kit includes an elongation oligonucleotide, it also often comprises chain elongating nucleotides, such as dATP, dTTP, dGTP, dCTP, and dITP, including analogs of dATP, dTTP, dGTP, dCTP and dITP, provided that such analogs are substrates for a thermostable nucleic acid polymerase and can be incorporated into a nucleic acid chain elongated from the extension oligonucleotide. Along with chain elongating nucleotides would be one or more chain terminating nucleotides such as ddATP, ddTTP, ddGTP, ddCTP, and the like. In an embodiment, the kit comprises one or more oligonucleotide primer pairs, a polymerizing agent, chain elongating nucleotides, at least one elongation oligonucleotide, and one or more chain terminating nucleotides. Kits optionally include buffers, vials, microtiter plates, and instructions for use.

[0103] An individual identified as being at risk of osteoporosis may be heterozygous or homozygous with respect to the allele associated with low BMD. A subject homozygous for an allele associated with low BMD is at a comparatively high risk of osteoporosis, a subject heterozygous for an allele associated with low BMD is at a comparatively intermediate risk of osteoporosis, and a subject homozygous for an allele associated with normal or high BMD levels (*i.e.*, a decreased risk of low BMD) is at a comparatively low risk of osteoporosis. A genotype may be assessed for a complementary strand, such that the complementary nucleotide at a particular position is detected.

[0104] Also featured are methods for determining risk of osteoporosis and/or identifying a subject at risk of osteoporosis by contacting a polypeptide or protein encoded by a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence from a subject with an antibody that specifically binds to an epitope associated with increased risk of osteoporosis in the polypeptide. In an embodiment, the antibody specifically binds to an epitope comprising an arginine at position 120 in a *PROL4* polypeptide.

Applications of Prognostic and Diagnostic Results to Pharmacogenomic Methods

[0105] Pharmacogenomics is a discipline that involves tailoring a treatment for a subject according to the subject's genotype as a particular treatment regimen may exert a differential effect depending upon the subject's genotype. For example, based upon the outcome of a prognostic test described herein, a clinician or physician may target pertinent information and preventative or therapeutic treatments to a subject who would be benefited by the information or treatment and avoid

directing such information and treatments to a subject who would not be benefited (*e.g.*, the treatment has no therapeutic effect and/or the subject experiences adverse side effects).

[0106] The following is an example of a pharmacogenomic embodiment. A particular treatment regimen can exert a differential effect depending upon the subject's genotype. Where a candidate therapeutic exhibits a significant interaction with a major allele and a comparatively weak interaction with a minor allele (*e.g.*, an order of magnitude or greater difference in the interaction), such a therapeutic typically would not be administered to a subject genotyped as being homozygous for the minor allele, and sometimes not administered to a subject genotyped as being heterozygous for the minor allele. In another example, where a candidate therapeutic is not significantly toxic when administered to subjects who are homozygous for a major allele but is comparatively toxic when administered to subjects heterozygous or homozygous for a minor allele, the candidate therapeutic is not typically administered to subjects who are genotyped as being heterozygous or homozygous with respect to the minor allele.

[0107] The methods described herein are applicable to pharmacogenomic methods for preventing, alleviating or treating low BMD conditions such as osteoporosis. For example, a nucleic acid sample from an individual may be subjected to a prognostic test described herein. Where one or more polymorphic variations associated with low BMD are identified in a subject, information for preventing or treating osteoporosis and/or one or more osteoporosis treatment regimens then may be prescribed to that subject.

[0108] In certain embodiments, a treatment or preventative regimen is specifically prescribed and/or administered to individuals who will most benefit from it based upon their risk of developing osteoporosis assessed by the methods described herein. Thus, provided are methods for identifying a subject predisposed to osteoporosis and then prescribing a therapeutic or preventative regimen to individuals identified as having a predisposition. Thus, certain embodiments are directed to a method for increasing BMD levels or otherwise treating osteoporosis in a subject, which comprises: detecting the presence or absence of a polymorphic variant associated with low BMD in a nucleotide sequence in a nucleic acid sample from a subject, where the nucleotide sequence comprises a polynucleotide sequence selected from the group consisting of: (a) a nucleotide sequence of SEQ ID NO's:1-10; (b) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-10; (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-10, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO's:1-10; and (d) a fragment of a polynucleotide sequence of (a), (b), or (c); and prescribing or administering a treatment regimen to a subject from whom the sample originated where the presence of a polymorphic variation associated with low BMD is detected in the nucleotide sequence. In these methods, predisposition results may be utilized in combination with other test results to diagnose low BMD associated conditions, such as osteoporosis.

[0109] Certain preventative treatments often are prescribed to subjects having a predisposition to osteoporosis and where the subject is diagnosed with low BMD or is diagnosed as having symptoms indicative of early stage osteoporosis. Established diagnostic techniques use x-ray and ultrasonography to measure skeletal parameters of bone size, volume and mineral density to predict fracture risk and to assess response to therapy. Such measurements give a "static" value which can be compared to normal values to aid diagnosis of low bone mass and fracture risk (Schott, Cornier et al. 1998). The World Health Organization defines osteoporosis as a bone mineral density level more than 2.5 standard deviations below the young normal mean. The various techniques used to measure bone mineral density include, a) Dual energy X-ray absorptiometry (DXA) - used to measure bone mass at the lumbar spine and hip, but it can also be applied to measuring total skeletal bone mass, soft-tissue composition and other regional bone measurements. DXA is considered the "gold standard" for BMD measurement; b) high-resolution quantitative computed tomography QCT) - highly sensitive, accurate and specific spinal measurements. This technique is more costly, not widely available and involves higher radiation doses than other techniques; c) single-energy x-ray absorptiometry (SXA) - provides accurate radius BMD measurements; d) quantitative ultrasound QUS) - new and promising technique which may have applications in both BMD measurement and assessment or architectural deterioration of bone tissue. Recent studies suggest QUS of calcaneus bone predicts hip fracture as well as DXA (Hans, Dargent-Molina et al. 1996).

[0110] An alternative method to predict fracture independently of bone mass is to measure bone turnover. High turnover (bone resorption and formation) is associated with rapid bone loss and is likely to contribute to micro-architectural deterioration (Ross and Knowlton 1998). This is a "dynamic" measurement which is assessed with biochemical markers in urine or serum and can be used effectively in therapy monitoring in preference to BNO measurements. When used in combination with bone mass assessment, biomarkers can provide more accurate fracture predictions over bone mass measurement alone. Osteoporosis-related biomarkers for bone resorption include deoxypyridinoline crosslinks, and osteoporosis-related biomarkers for bone formation include bone alkaline phosphatase and osteocalcin. Some of these biomarkers have been developed for use in diagnostic kits. The current challenge is to reduce the variability of the measurements and improve their reliability and applicability.

[0111] The treatment sometimes is preventative (*e.g.*, is prescribed or administered to reduce the probability that a low BMD associated condition arises or progresses), sometimes is therapeutic, and sometimes delays, alleviates or halts the progression of a low BMD associated condition. Any known preventative or therapeutic treatment for alleviating or preventing the occurrence of a low BMD associated disorder is prescribed and/or administered. Preventative treatment for osteoporosis is most effective at the time when bone loss is increasing and before the bones have become fragile and prone to fracturing. Strategies for the prevention of this disease include development of bone density in early adulthood (*i.e.*, building strong bones during childhood and adolescence), and minimization of bone loss in later life. Changes in lifestyle, nutrition and hormonal factors have been shown to affect bone

loss. Specifically, a balanced diet rich in calcium and vitamin D, weight-bearing exercise, a healthy lifestyle with no smoking or excessive alcohol intake, and bone density testing and medication when appropriate are known to help reduce the risk of osteoporosis.

[0112] As therapeutic approaches for low BMD continue to evolve and improve, the goal of treatments for low BMD related disorders is to intervene even before clinical signs (*e.g.*, impaired glucose tolerance, or IGT) first manifest. Thus, genetic markers associated with susceptibility to low BMD prove useful for early diagnosis, prevention and treatment of low BMD.

[0113] As osteoporosis preventative and treatment information can be specifically targeted to subjects in need thereof (*e.g.*, those at risk of low BMD or those that have early stages of osteoporosis), provided herein is a method for preventing or reducing the risk of developing osteoporosis in a subject, which comprises: (a) detecting the presence or absence of a polymorphic variation associated with low BMD at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject; (b) identifying a subject with a predisposition to osteoporosis, whereby the presence of the polymorphic variation is indicative of a predisposition to osteoporosis in the subject; and (c) if such a predisposition is identified, providing the subject with information about methods or products to prevent osteoporosis or to delay the onset of osteoporosis. Also provided is a method of targeting information or advertising to a subpopulation of a human population based on the subpopulation being genetically predisposed to a disease or condition, which comprises: (a) detecting the presence or absence of a polymorphic variation associated with low BMD at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject; (b) identifying the subpopulation of subjects in which the polymorphic variation is associated with low BMD; and (c) providing information only to the subpopulation of subjects about a particular product which may be obtained and consumed or applied by the subject to help prevent or delay onset of the disease or condition.

[0114] Pharmacogenomics methods also may be used to analyze and predict a response to a osteoporosis treatment or a drug. For example, if pharmacogenomics analysis indicates a likelihood that an individual will respond positively to a osteoporosis treatment with a particular drug, the drug may be administered to the individual. Conversely, if the analysis indicates that an individual is likely to respond negatively to treatment with a particular drug, an alternative course of treatment may be prescribed. A negative response may be defined as either the absence of an efficacious response or the presence of toxic side effects. The response to a therapeutic treatment can be predicted in a background study in which subjects in any of the following populations are genotyped: a population that responds favorably to a treatment regimen, a population that does not respond significantly to a treatment regimen, and a population that responds adversely to a treatment regiment (*e.g.*, exhibits one or more side effects). These populations are provided as examples and other populations and subpopulations may be analyzed. Based upon the results of these analyses, a subject is genotyped to predict whether he or she will respond favorably to a treatment regimen, not respond significantly to a treatment regimen, or respond adversely to a treatment regimen.

[0115] The tests described herein also are applicable to clinical drug trials. One or more polymorphic variants indicative of response to an agent for treating osteoporosis or to side effects to an agent for treating osteoporosis may be identified using the methods described herein. Thereafter, potential participants in clinical trials of such an agent may be screened to identify those individuals most likely to respond favorably to the drug and exclude those likely to experience side effects. In that way, the effectiveness of drug treatment may be measured in individuals who respond positively to the drug, without lowering the measurement as a result of the inclusion of individuals who are unlikely to respond positively in the study and without risking undesirable safety problems.

[0116] Thus, another embodiment is a method of selecting an individual for inclusion in a clinical trial of a treatment or drug comprising the steps of: (a) obtaining a nucleic acid sample from an individual; (b) determining the identity of a polymorphic variation which is associated with a positive response to the treatment or the drug, or at least one polymorphic variation which is associated with a negative response to the treatment or the drug in the nucleic acid sample, and (c) including the individual in the clinical trial if the nucleic acid sample contains said polymorphic variation associated with a positive response to the treatment or the drug or if the nucleic acid sample lacks said polymorphic variation associated with a negative response to the treatment or the drug. In addition, the methods described herein for selecting an individual for inclusion in a clinical trial of a treatment or drug encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination. The polymorphic variation may be in a sequence selected individually or in any combination from the group consisting of (i) a nucleotide sequence of SEQ ID NO's:1-10; (ii) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-10; (iii) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-10, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO's:1-10; and (iv) a fragment of a polynucleotide sequence of (i), (ii), or (iii) comprising the polymorphic site. The including step (c) optionally comprises administering the drug or the treatment to the individual if the nucleic acid sample contains the polymorphic variation associated with a positive response to the treatment or the drug and the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug.

[0117] Also provided herein is a method of partnering between a diagnostic/prognostic testing provider and a provider of a consumable product, which comprises: (a) the diagnostic/prognostic testing provider detects the presence or absence of a polymorphic variation associated with low BMD at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject; (b) the diagnostic/prognostic testing provider identifies the subpopulation of subjects in which the polymorphic variation is associated with low BMD; (c) the diagnostic/prognostic testing provider forwards information to the subpopulation of subjects about a particular product which may be obtained and consumed or applied by the subject to help prevent or delay onset of the disease or condition; and

(d) the provider of a consumable product forwards to the diagnostic test provider a fee every time the diagnostic/prognostic test provider forwards information to the subject as set forth in step (c) above.

Compositions Comprising Osteoporosis-Directed Molecules

[0118] Featured herein is a composition comprising a cell from a subject having low BMD or at risk of osteoporosis and one or more molecules specifically directed and targeted to a nucleic acid comprising a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence or amino acid sequence. Such directed molecules include, but are not limited to, a compound that binds to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence or amino acid sequence referenced herein; a nucleic acid that hybridizes to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid under conditions of high stringency; a RNAi or siRNA molecule having a strand complementary to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence; an antisense nucleic acid complementary to an RNA encoded by a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence; a ribozyme that hybridizes to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence; a nucleic acid aptamer that specifically binds a polypeptide encoded by *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence; and an antibody that specifically binds to a polypeptide encoded by *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence or binds to a nucleic acid having such a nucleotide sequence. In specific embodiments, the osteoporosis directed molecule interacts with a nucleic acid or polypeptide variant associated with osteoporosis, such as variants referenced herein. In other embodiments, the osteoporosis directed molecule interacts with a polypeptide involved in a signal pathway of a polypeptide encoded by a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence, or a nucleic acid comprising such a nucleotide sequence.

[0119] Compositions sometimes include an adjuvant known to stimulate an immune response, and in certain embodiments, an adjuvant that stimulates a T-cell lymphocyte response. Adjuvants are known, including but not limited to an aluminum adjuvant (*e.g.*, aluminum hydroxide); a cytokine adjuvant or adjuvant that stimulates a cytokine response (*e.g.*, interleukin (IL)-12 and/or γ-interferon cytokines); a Freund-type mineral oil adjuvant emulsion (*e.g.*, Freund's complete or incomplete adjuvant); a synthetic lipoid compound; a copolymer adjuvant (*e.g.*, TitreMax); a saponin; Quil A; a liposome; an oil-in-water emulsion (*e.g.*, an emulsion stabilized by Tween 80 and pluronic polyoxyethlene/polyoxypropylene block copolymer (Syntex Adjuvant Formulation); TitreMax; detoxified endotoxin (MPL) and mycobacterial cell wall components (TDW, CWS) in 2% squalene (Ribi Adjuvant System)); a muramyl dipeptide; an immune-stimulating complex (ISCOM, *e.g.*, an Ag-modified saponin/cholesterol micelle that forms stable cage-like structure); an aqueous phase adjuvant that does not have a depot effect (*e.g.*, Gerbu adjuvant); a carbohydrate polymer (*e.g.*, AdjuPrime); L-tyrosine; a manide-oleate compound (*e.g.*, Montanide); an ethylene-vinyl acetate copolymer (*e.g.*, Elvax 40W1,2); or lipid A, for example. Such compositions are useful for generating an immune response against a osteoporosis directed molecule (*e.g.*, an HLA-binding subsequence within a

polypeptide encoded by a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence). In such methods, a peptide having an amino acid subsequence of a polypeptide encoded by a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence is delivered to a subject, where the subsequence binds to an HLA molecule and induces a CTL lymphocyte response. The peptide sometimes is delivered to the subject as an isolated peptide or as a minigene in a plasmid that encodes the peptide. Methods for identifying HLA-binding subsequences in such polypeptides are known (see *e.g.*, publication WO02/20616 and PCT application US98/01373 for methods of identifying such sequences).

[0120] The cell may be in a group of cells cultured *in vitro* or in a tissue maintained *in vitro* or present in an animal *in vivo* (*e.g.*, a rat, mouse, ape or human). In certain embodiments, a composition comprises a component from a cell such as a nucleic acid molecule (*e.g.*, genomic DNA), a protein mixture or isolated protein, for example. The aforementioned compositions have utility in diagnostic, prognostic and pharmacogenomic methods described previously and in osteoporosis therapeutics described hereafter. Certain osteoporosis directed molecules are described in greater detail below.

Compounds

[0121] Compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive (see, *e.g.*, Zuckermann *et al.*, J. Med. Chem.37: 2678-85 (1994)); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; "one-bead one-compound" library methods; and synthetic library methods using affinity chromatography selection. Biological library and peptoid library approaches are typically limited to peptide libraries, while the other approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, Anticancer Drug Des. 12: 145, (1997)). Examples of methods for synthesizing molecular libraries are described, for example, in DeWitt *et al.*, Proc. Natl. Acad. Sci. U.S.A. 90: 6909 (1993); Erb *et al.*, Proc. Natl. Acad. Sci. USA 91: 11422 (1994); Zuckermann *et al.*, J. Med. Chem. 37: 2678 (1994); Cho *et al.*, Science 261: 1303 (1993); Carrell *et al.*, Angew. Chem. Int. Ed. Engl. 33: 2059 (1994); Carell *et al.*, Angew. Chem. Int. Ed. Engl. 33: 2061 (1994); and in Gallop *et al.*, J. Med. Chem. 37: 1233 (1994).

[0122] Libraries of compounds may be presented in solution (*e.g.*, Houghten, Biotechniques 13: 412-421 (1992)), or on beads (Lam, Nature 354: 82-84 (1991)), chips (Fodor, Nature 364: 555-556 (1993)), bacteria or spores (Ladner, United States Patent No. 5,223,409), plasmids (Cull *et al.*, Proc. Natl. Acad. Sci. USA 89: 1865-1869 (1992)) or on phage (Scott and Smith, Science 249: 386-390 (1990); Devlin, Science 249: 404-406 (1990); Cwirla *et al.*, Proc. Natl. Acad. Sci. 87: 6378-6382 (1990); Felici, J. Mol. Biol. 222: 301-310 (1991); Ladner supra.).

[0123] A compound sometimes alters expression and sometimes alters activity of a polypeptide target and may be a small molecule. Small molecules include, but are not limited to, peptides, peptidomimetics (*e.g.*, peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

Antisense Nucleic Acid Molecules, Ribozymes, RNAi, siRNA and Modified Nucleic Acid Molecules

[0124] An "antisense" nucleic acid refers to a nucleotide sequence complementary to a "sense" nucleic acid encoding a polypeptide, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire coding strand (*e.g.*, SEQ ID NO: 7-13), or to a portion thereof or a substantially identical sequence thereof. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence (*e.g.*, 5' and 3' untranslated regions in SEQ ID NO's:1-5).

[0125] An antisense nucleic acid can be designed such that it is complementary to the entire coding region of an mRNA encoded by a nucleotide sequence (*e.g.*, SEQ ID NO's:1-10), and often the antisense nucleic acid is an oligonucleotide antisense to only a portion of a coding or noncoding region of the mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of the mRNA, *e.g.*, between the -10 and +10 regions of the target gene nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length. The antisense nucleic acids, which include the ribozymes described hereafter, can be designed to target a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence, often a variant associated with low BMD, or a substantially identical sequence thereof. Among the variants, minor alleles and major alleles can be targeted, and those associated with a higher risk of osteoporosis are often designed, tested, and administered to subjects.

[0126] An antisense nucleic acid can be constructed using chemical synthesis and enzymatic ligation reactions using standard procedures. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Antisense nucleic acid

also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

[0127] When utilized as therapeutics, antisense nucleic acids typically are administered to a subject (*e.g.*, by direct injection at a tissue site) or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a polypeptide and thereby inhibit expression of the polypeptide, for example, by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then are administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, for example, by linking antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. Antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. Sufficient intracellular concentrations of antisense molecules are achieved by incorporating a strong promoter, such as a pol II or pol III promoter, in the vector construct.

[0128] Antisense nucleic acid molecules sometimes are *-anomeric nucleic acid molecules. An *-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual *-units, the strands run parallel to each other (Gaultier *et al.*, Nucleic Acids. Res. 15: 6625-6641 (1987)). Antisense nucleic acid molecules can also comprise a 2'-o-methylribonucleotide (Inoue *et al.*, Nucleic Acids Res. 15: 6131-6148 (1987)) or a chimeric RNA-DNA analogue (Inoue *et al.*, FEBS Lett. 215: 327-330 (1987)). Antisense nucleic acids sometimes are composed of DNA or PNA or any other nucleic acid derivatives described previously.

[0129] In another embodiment, an antisense nucleic acid is a ribozyme. A ribozyme having specificity for a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence can include one or more sequences complementary to such a nucleotide sequence, and a sequence having a known catalytic region responsible for mRNA cleavage (see *e.g.*, U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach, Nature 334: 585-591 (1988)). For example, a derivative of a Tetrahymena L-19 IVS RNA is sometimes utilized in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a mRNA (see *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742). Also, target mRNA sequences can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (see *e.g.*, Bartel & Szostak, Science 261: 1411-1418 (1993)).

[0130] Osteoporosis directed molecules include in certain embodiments nucleic acids that can form triple helix structures with a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence or a substantially identical sequence thereof, especially one that includes a regulatory region that controls expression of a polypeptide. Gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of a nucleotide sequence referenced herein or a substantially identical sequence (*e.g.*, promoter and/or enhancers) to form triple helical structures that

prevent transcription of a gene in target cells (see *e.g.*, Helene, Anticancer Drug Des. 6(6): 569-84 (1991); Helene *et al.*, Ann. N.Y. Acad. Sci. 660: 27-36 (1992); and Maher, Bioassays 14(12): 807-15 (1992). Potential sequences that can be targeted for triple helix formation can be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

[0131] Osteoporosis directed molecules include RNAi and siRNA nucleic acids. Gene expression may be inhibited by the introduction of double-stranded RNA (dsRNA), which induces potent and specific gene silencing, a phenomenon called RNA interference or RNAi. See, *e.g.*, Fire *et al.*, US Patent Number 6,506,559; Tuschl *et al.* PCT International Publication No. WO 01/75164; Kay *et al.* PCT International Publication No. WO 03/010180A1; or Bosher JM, Labouesse, Nat Cell Biol 2000 Feb;2(2):E31-6. This process has been improved by decreasing the size of the double-stranded RNA to 20-24 base pairs (to create small-interfering RNAs or siRNAs) that "switched off" genes in mammalian cells without initiating an acute phase response, *i.e.*, a host defense mechanism that often results in cell death (see, *e.g.*, Caplen *et al.* Proc Natl Acad Sci U S A. 2001 Aug 14;98(17):9742-7 and Elbashir *et al.* Methods 2002 Feb;26(2):199-213). There is increasing evidence of post-transcriptional gene silencing by RNA interference (RNAi) for inhibiting targeted expression in mammalian cells at the mRNA level, in human cells. There is additional evidence of effective methods for inhibiting the proliferation and migration of tumor cells in human patients, and for inhibiting metastatic cancer development (see, *e.g.*, U.S. Patent Application No. US2001000993183; Caplen *et al.* Proc Natl Acad Sci U S A; and Abderrahmani *et al.* Mol Cell Biol 2001 Nov 21(21):7256-67).

[0132] An "siRNA" or "RNAi" refers to a nucleic acid that forms a double stranded RNA and has the ability to reduce or inhibit expression of a gene or target gene when the siRNA is delivered to or expressed in the same cell as the gene or target gene. "siRNA" refers to short double-stranded RNA formed by the complementary strands. Complementary portions of the siRNA that hybridize to form the double stranded molecule often have substantial or complete identity to the target molecule sequence. In one embodiment, an siRNA refers to a nucleic acid that has substantial or complete identity to a target gene and forms a double stranded siRNA.

[0133] When designing the siRNA molecules, the targeted region often is selected from a given DNA sequence beginning 50 to 100 nucleotides downstream of the start codon. See, *e.g.*, Elbashir *et al*,. Methods 26:199-213 (2002). Initially, 5' or 3' UTRs and regions nearby the start codon were avoided assuming that UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNP or RISC endonuclease complex. Sometimes regions of the target 23 nucleotides in length conforming to the sequence motif AA(N19)TT (N, an nucleotide), and regions with approximately 30% to 70% G/C-content (often about 50% G/C-content) often are selected. If no suitable sequences are found, the search often is extended using the motif NA(N21). The sequence of

the sense siRNA sometimes corresponds to (N19) TT or N21 (position 3 to 23 of the 23-nt motif), respectively. In the latter case, the 3' end of the sense siRNA often is converted to TT. The rationale for this sequence conversion is to generate a symmetric duplex with respect to the sequence composition of the sense and antisense 3' overhangs. The antisense siRNA is synthesized as the complement to position 1 to 21 of the 23-nt motif. Because position 1 of the 23-nt motif is not recognized sequence-specifically by the antisense siRNA, the 3'-most nucleotide residue of the antisense siRNA can be chosen deliberately. However, the penultimate nucleotide of the antisense siRNA (complementary to position 2 of the 23-nt motif) often is complementary to the targeted sequence. For simplifying chemical synthesis, TT often is utilized. siRNAs corresponding to the target motif NAR(N17)YNN, where R is purine (A,G) and Y is pyrimidine (C,U), often are selected. Respective 21 nucleotide sense and antisense siRNAs often begin with a purine nucleotide and can also be expressed from pol III expression vectors without a change in targeting site. Expression of RNAs from pol III promoters often is efficient when the first transcribed nucleotide is a purine.

[0134] The sequence of the siRNA can correspond to the full length target gene, or a subsequence thereof. Often, the siRNA is about 15 to about 50 nucleotides in length (*e.g.*, each complementary sequence of the double stranded siRNA is 15-50 nucleotides in length, and the double stranded siRNA is about 15-50 base pairs in length, sometimes about 20-30 nucleotides in length or about 20-25 nucleotides in length, *e.g.*, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. The siRNA sometimes is about 21 nucleotides in length. Methods of using siRNA are well known in the art, and specific siRNA molecules may be purchased from a number of companies including Dharmacon Research, Inc. An siRNA molecule sometimes is of a different chemical composition as compared to native RNA that imparts increased stability in cells (*e.g.*, decreased susceptibility to degradation), and sometimes includes one or more modifications in siSTABLE RNA described at the http address www.dharmacon.com.

[0135] Antisense, ribozyme, RNAi and siRNA nucleic acids can be altered to form modified nucleic acid molecules. The nucleic acids can be altered at base moieties, sugar moieties or phosphate backbone moieties to improve stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup *et al.*, Bioorganic & Medicinal Chemistry 4 (1): 5-23 (1996)). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic such as a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. Synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described, for example, in Hyrup *et al.*, (1996) supra and Perry-O'Keefe *et al.*, Proc. Natl. Acad. Sci. 93: 14670-675 (1996).

[0136] PNA nucleic acids can be used in prognostic, diagnostic, and therapeutic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene

expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNA nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as "artificial restriction enzymes" when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup (1996) supra)); or as probes or primers for DNA sequencing or hybridization (Hyrup *et al.*, (1996) supra; Perry-O'Keefe supra).

[0137] In other embodiments, oligonucleotides may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors in vivo), or agents facilitating transport across cell membranes (see *e.g.*, Letsinger *et al.*, Proc. Natl. Acad. Sci. USA 86: 6553-6556 (1989); Lemaitre *et al.*, Proc. Natl. Acad. Sci. USA 84: 648-652 (1987); PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W0 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.*, Bio-Techniques 6: 958-976 (1988)) or intercalating agents. (See, *e.g.*, Zon, Pharm. Res. 5: 539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

[0138] Also included herein are molecular beacon oligonucleotide primer and probe molecules having one or more regions complementary to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence or a substantially identical sequence thereof, two complementary regions one having a fluorophore and one a quencher such that the molecular beacon is useful for quantifying the presence of the nucleic acid in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi *et al.*, U.S. Patent No. 5,854,033; Nazarenko *et al.*, U.S. Patent No. 5,866,336, and Livak *et al.*, U.S. Patent 5,876,930.

Antibody

[0139] The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, *i.e.*, an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. An antibody sometimes is a polyclonal, monoclonal, recombinant (*e.g.*, a chimeric or humanized), fully human, non-human (*e.g.*, murine), or a single chain antibody. An antibody may have effector function and can fix complement, and is sometimes coupled to a toxin or imaging agent.

Antibodies

[0140] A full-length polypeptide or antigenic peptide fragment encoded by a nucleotide sequence referenced herein can be used as an immunogen or can be used to identify antibodies made with other immunogens, *e.g.*, cells, membrane preparations, and the like. An antigenic peptide often includes at least 8 amino acid residues of the amino acid sequences encoded by a nucleotide sequence referenced herein, or substantially identical sequence thereof, and encompasses an epitope. Antigenic peptides

sometimes include 10 or more amino acids, 15 or more amino acids, 20 or more amino acids, or 30 or more amino acids. Hydrophilic and hydrophobic fragments of polypeptides sometimes are used as immunogens.

[0141] Epitopes encompassed by the antigenic peptide are regions located on the surface of the polypeptide (*e.g.*, hydrophilic regions) as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human polypeptide sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the polypeptide and are thus likely to constitute surface residues useful for targeting antibody production. The antibody may bind an epitope on any domain or region on polypeptides described herein.

[0142] Also, chimeric, humanized, and completely human antibodies are useful for applications which include repeated administration to subjects. Chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, can be made using standard recombinant DNA techniques. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al* International Application No. PCT/US86/02269; Akira, *et al* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al* European Patent Application 173,494; Neuberger *et al* PCT International Publication No. WO 86/01533; Cabilly *et al* U.S. Patent No. 4,816,567; Cabilly *et al* European Patent Application 125,023; Better *et al.*, Science 240: 1041-1043 (1988); Liu *et al.*, Proc. Natl. Acad. Sci. USA 84: 3439-3443 (1987); Liu *et al.*, J. Immunol. 139: 3521-3526 (1987); Sun *et al.*, Proc. Natl. Acad. Sci. USA 84: 214-218 (1987); Nishimura *et al.*, Canc. Res. 47: 999-1005 (1987); Wood *et al.*, Nature 314: 446-449 (1985); and Shaw *et al.*, J. Natl. Cancer Inst. 80: 1553-1559 (1988); Morrison, S. L., Science 229: 1202-1207 (1985); Oi *et al.*, BioTechniques 4: 214 (1986); Winter U.S. Patent 5,225,539; Jones *et al.*, Nature 321: 552-525 (1986); Verhoeyan *et al.*, Science 239: 1534; and Beidler *et al.*, J. Immunol. 141: 4053-4060 (1988).

[0143] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. See, for example, Lonberg and Huszar, Int. Rev. Immunol. 13: 65-93 (1995); and U.S. Patent Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806. In addition, companies such as Abgenix, Inc. (Fremont, CA) and Medarex, Inc. (Princeton, NJ), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above. Completely human antibodies that recognize a selected epitope also can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody (*e.g.*, a murine antibody) is used to guide the selection of a completely human antibody recognizing the same epitope. This technology is described for example by Jespers *et al.*, Bio/Technology 12: 899-903 (1994).

[0144] An antibody can be a single chain antibody. A single chain antibody (scFV) can be engineered (see, *e.g.*, Colcher *et al.*, Ann. N Y Acad. Sci. 880: 263-80 (1999); and Reiter, Clin. Cancer Res. 2: 245-52 (1996)). Single chain antibodies can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target polypeptide.

[0145] Antibodies also may be selected or modified so that they exhibit reduced or no ability to bind an Fc receptor. For example, an antibody may be an isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor (*e.g.*, it has a mutagenized or deleted Fc receptor binding region).

[0146] Also, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1 dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thiotepa chlorambucil, melphalan, carmustine (BCNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[0147] Antibody conjugates can be used for modifying a given biological response. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide such as tumor necrosis factor, γ-interferon, α-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Also, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, for example.

[0148] An antibody (*e.g.*, monoclonal antibody) can be used to isolate target polypeptides by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an antibody can be used to detect a target polypeptide (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. Antibodies can be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance (*i.e.*, antibody labeling). Examples of

detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H. Also, an antibody can be utilized as a test molecule for determining whether it can treat osteoporosis, and as a therapeutic for administration to a subject for treating osteoporosis.

[0149] An antibody can be made by immunizing with a purified antigen, or a fragment thereof, *e.g.*, a fragment described herein, a membrane associated antigen, tissues, *e.g.*, crude tissue preparations, whole cells, preferably living cells, lysed cells, or cell fractions.

[0150] Included herein are antibodies which bind only a native polypeptide, only denatured or otherwise non-native polypeptide, or which bind both, as well as those having linear or conformational epitopes. Conformational epitopes sometimes can be identified by selecting antibodies that bind to native but not denatured polypeptide. Also featured are antibodies that specifically bind to a polypeptide variant associated with low BMD.

Methods for Identifying Candidate Therapeutics for Treating Osteoporosis

[0151] Current treatment for osteoporosis can help stop further bone loss and fractures, but there is still a clear need for specific anabolic agents that considerably increase bone formation in people who have already suffered substantial bone loss. There are no such drugs currently approved. Known osteoporosis treatments include bone-active phosphonates (See, *e.g.*, U.S. Patent No. 6,329,354), bisphosphonates, *e.g.*, alendronate (Fosamax®) and risedronate (Actonel®); calcitonin (Miacalcin®); estrogen and hormone therapy, *e.g.*, estrogens (Climara®, Estrace®, Estraderm®, Estratab®, Ogen®, Ortho-Est®, Vivelle®, Premarin®, and others) and estrogens and progestins (brand names, such as Activella™, FemHrt®, Premphase®, Prempro®, and others); parathyroid hormone, *e.g.*, teriparatide {PTH 1-34} (Forteo®); as well as, estrogen and estrogen receptor modulators (SERMs), *e.g.*, raloxifene HCl (Evista®), sodium fluoride and vitamin D metabolites. Any of the above therapeutics may be administered alone or in combination (e.g., bone-active phosphonates and estrogen and hormone therapy). Current therapeutic approaches were largely developed in the absence of defined molecular targets or even a solid understanding of disease pathogenesis. Therefore, provided are methods of identifying candidate therapeutics that target biochemical pathways related to the development of osteoporosis.

[0152] Thus, featured herein are methods for identifying a candidate therapeutic for treating osteoporosis. The methods comprise contacting a test molecule with a target molecule in a system. A

"target molecule" as used herein refers to a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid, a substantially identical nucleic acid thereof, or a fragment thereof, and an encoded polypeptide of the foregoing. The methods also comprise determining the presence or absence of an interaction between the test molecule and the target molecule, where the presence of an interaction between the test molecule and the nucleic acid or polypeptide identifies the test molecule as a candidate osteoporosis therapeutic. The interaction between the test molecule and the target molecule may be quantified.

[0153] Test molecules and candidate therapeutics include, but are not limited to, compounds, antisense nucleic acids, siRNA molecules, ribozymes, polypeptides or proteins encoded by a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleotide sequence, or a substantially identical sequence or fragment thereof, and immunotherapeutics (*e.g.*, antibodies and HLA-presented polypeptide fragments). A test molecule or candidate therapeutic may act as a modulator of target molecule concentration or target molecule function in a system. A "modulator" may agonize (*i.e.*, up-regulates) or antagonize (*i.e.*, down-regulates) a target molecule concentration partially or completely in a system by affecting such cellular functions as DNA replication and/or DNA processing (*e.g.*, DNA methylation or DNA repair), RNA transcription and/or RNA processing (*e.g.*, removal of intronic sequences and/or translocation of spliced mRNA from the nucleus), polypeptide production (*e.g.*, translation of the polypeptide from mRNA), and/or polypeptide post-translational modification (*e.g.*, glycosylation, phosphorylation, and proteolysis of pro-polypeptides). A modulator may also agonize or antagonize a biological function of a target molecule partially or completely, where the function may include adopting a certain structural conformation, interacting with one or more binding partners, ligand binding, catalysis (*e.g.*, phosphorylation, dephosphorylation, hydrolysis, methylation, and isomerization), and an effect upon a cellular event (*e.g.*, effecting progression of osteoporosis). In certain embodiments, a candidate therapeutic increases BMD.

[0154] As used herein, the term "system" refers to a cell free in vitro environment and a cell-based environment such as a collection of cells, a tissue, an organ, or an organism. A system is "contacted" with a test molecule in a variety of manners, including adding molecules in solution and allowing them to interact with one another by diffusion, cell injection, and any administration routes in an animal. As used herein, the term "interaction" refers to an effect of a test molecule on test molecule, where the effect sometimes is binding between the test molecule and the target molecule, and sometimes is an observable change in cells, tissue, or organism.

[0155] There are many standard methods for detecting the presence or absence of interaction between a test molecule and a target molecule. For example, titrametric, acidimetric, radiometric, NMR, monolayer, polarographic, spectrophotometric, fluorescent, and ESR assays probative of a target molecule interaction may be utilized.

[0156] Test molecule/target molecule interactions can be detected and/or quantified using assays known in the art. For example, an interaction can be determined by labeling the test molecule and/or

the target molecule, where the label is covalently or non-covalently attached to the test molecule or target molecule. The label is sometimes a radioactive molecule such as ^{125}I, ^{131}I, ^{35}S or ^{3}H, which can be detected by direct counting of radioemission or by scintillation counting. Also, enzymatic labels such as horseradish peroxidase, alkaline phosphatase, or luciferase may be utilized where the enzymatic label can be detected by determining conversion of an appropriate substrate to product. In addition, presence or absence of an interaction can be determined without labeling. For example, a microphysiometer (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indication of an interaction between a test molecule and target molecule (McConnell, H. M. *et al., Science 257:* 1906-1912 (1992)).

[0157] In cell-based systems, cells typically include a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid, an encoded polypeptide, or substantially identical nucleic acid or polypeptide thereof, and are often of mammalian origin, although the cell can be of any origin. Whole cells, cell homogenates, and cell fractions (*e.g.*, cell membrane fractions) can be subjected to analysis. Where interactions between a test molecule with a target polypeptide are monitored, soluble and/or membrane bound forms of the polypeptide may be utilized. Where membrane-bound forms of the polypeptide are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate.

[0158] An interaction between a test molecule and target molecule also can be detected by monitoring fluorescence energy transfer (FET) (*see, e.g.*, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos *et al.* U.S. Patent No. 4,868,103). A fluorophore label on a first, "donor" molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, "acceptor" molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the "donor" polypeptide molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the "acceptor" molecule label may be differentiated from that of the "donor". Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the "acceptor" molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (*e.g.*, using a fluorimeter).

[0159] In another embodiment, determining the presence or absence of an interaction between a test molecule and a target molecule can be effected by monitoring surface plasmon resonance (*see, e.g.*,

Sjolander & Urbaniczk, *Anal. Chem. 63:* 2338-2345 (1991) and Szabo *et al., Curr. Opin. Struct. Biol. 5:* 699-705 (1995)). "Surface plasmon resonance" or "biomolecular interaction analysis (BIA)" can be utilized to detect biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

[0160] In another embodiment, the target molecule or test molecules are anchored to a solid phase, facilitating the detection of target molecule/test molecule complexes and separation of the complexes from free, uncomplexed molecules. The target molecule or test molecule is immobilized to the solid support. In an embodiment, the target molecule is anchored to a solid surface, and the test molecule, which is not anchored, can be labeled, either directly or indirectly, with detectable labels discussed herein.

[0161] It may be desirable to immobilize a target molecule, an anti-target molecule antibody, and/or test molecules to facilitate separation of target molecule/test molecule complexes from uncomplexed forms, as well as to accommodate automation of the assay. The attachment between a test molecule and/or target molecule and the solid support may be covalent or non-covalent (*see, e.g.*, U.S. Patent No. 6,022,688 for non-covalent attachments). The solid support may be one or more surfaces of the system, such as one or more surfaces in each well of a microtiter plate, a surface of a silicon wafer, a surface of a bead (*see, e.g.*, Lam, *Nature 354:* 82-84 (1991)) that is optionally linked to another solid support, or a channel in a microfluidic device, for example. Types of solid supports, linker molecules for covalent and non-covalent attachments to solid supports, and methods for immobilizing nucleic acids and other molecules to solid supports are well known (*see, e.g.*, U.S. Patent Nos. 6,261,776; 5,900,481; 6,133,436; and 6,022,688; and WIPO publication WO 01/18234).

[0162] In an embodiment, target molecule may be immobilized to surfaces via biotin and streptavidin. For example, biotinylated target polypeptide can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). In another embodiment, a target polypeptide can be prepared as a fusion polypeptide. For example, glutathione-S-transferase/target polypeptide fusion can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivitized microtiter plates, which are then combined with a test molecule under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, or the matrix is immobilized in the case of beads, and complex formation is determined directly or indirectly as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of target molecule binding or activity is determined using standard techniques.

[0163] In an embodiment, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that a significant percentage of complexes formed will remain immobilized to the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of manners. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface, *e.g.*, by adding a labeled antibody specific for the immobilized component, where the antibody, in turn, can be directly labeled or indirectly labeled with, *e.g.*, a labeled anti-Ig antibody.

[0164] In another embodiment, an assay is performed utilizing antibodies that specifically bind target molecule or test molecule but do not interfere with binding of the target molecule to the test molecule. Such antibodies can be derivitized to a solid support, and unbound target molecule may be immobilized by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the target molecule.

[0165] Cell free assays also can be conducted in a liquid phase. In such an assay, reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (*see, e.g.*, Rivas, G., and Minton, *Trends Biochem Sci Aug;18(8):* 284-7 (1993)); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (*see, e.g.*, Ausubel *et al., eds. Current Protocols in Molecular Biology , J. Wiley:* New York (1999)); and immunoprecipitation (*see, e.g.*, Ausubel *et al., eds., supra*). Media and chromatographic techniques are known to one skilled in the art (*see, e.g.*, Heegaard, *J Mol. Recognit. Winter; 11(1-6):* 141-8 (1998); Hage & Tweed, *J. Chromatogr. B Biomed. Sci. Appl. Oct 10; 699 (1-2): 499-525* (1997)). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

[0166] In another embodiment, modulators of target molecule expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of target mRNA or target polypeptide is evaluated relative to the level of expression of target mRNA or target polypeptide in the absence of the candidate compound. When expression of target mRNA or target polypeptide is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as an agonist of target mRNA or target polypeptide expression. Alternatively, when expression of target mRNA or target polypeptide is less (*e.g.*, less with statistical significance) in the presence of the candidate compound than in its absence, the candidate compound is identified as an antagonist or inhibitor of target mRNA or target polypeptide expression. The level of target mRNA or target polypeptide expression can be determined by methods described herein.

[0167] In another embodiment, binding partners that interact with a target molecule are detected. The target molecules can interact with one or more cellular or extracellular macromolecules, such as polypeptides *in vivo*, and these interacting molecules are referred to herein as "binding partners." Binding partners can agonize or antagonize target molecule biological activity. Also, test molecules that agonize or antagonize interactions between target molecules and binding partners can be useful as therapeutic molecules as they can up-regulate or down-regulated target molecule activity *in vivo* and thereby treat osteoporosis.

[0168] Binding partners of target molecules can be identified by methods known in the art. For example, binding partners may be identified by lysing cells and analyzing cell lysates by electrophoretic techniques. Alternatively, a two-hybrid assay or three-hybrid assay can be utilized (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos *et al., Cell 72:223-232* (1993); Madura *et al., J. Biol. Chem. 268:* 12046-12054 (1993); Bartel *et al., Biotechniques 14:* 920-924 (1993); Iwabuchi *et al., Oncogene 8:* 1693-1696 (1993); and Brent WO94/10300). A two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. The assay often utilizes two different DNA constructs. In one construct, a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid (sometimes referred to as the "bait") is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In another construct, a DNA sequence from a library of DNA sequences that encodes a potential binding partner (sometimes referred to as the "prey") is fused to a gene that encodes an activation domain of the known transcription factor. Sometimes, a *CETP, PROL4, GRID2, PDE4D* or *GPX3/TNIP1* nucleic acid can be fused to the activation domain. If the "bait" and the "prey" molecules interact *in vivo*, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to identify the potential binding partner.

[0169] In an embodiment for identifying test molecules that antagonize or agonize complex formation between target molecules and binding partners, a reaction mixture containing the target molecule and the binding partner is prepared, under conditions and for a time sufficient to allow complex formation. The reaction mixture often is provided in the presence or absence of the test molecule. The test molecule can be included initially in the reaction mixture, or can be added at a time subsequent to the addition of the target molecule and its binding partner. Control reaction mixtures are incubated without the test molecule or with a placebo. Formation of any complexes between the target molecule and the binding partner then is detected. Decreased formation of a complex in the reaction mixture containing test molecule as compared to in a control reaction mixture indicates that the molecule antagonizes target molecule/binding partner complex formation. Alternatively, increased formation of a complex in the reaction mixture containing test molecule as compared to in a control

reaction mixture indicates that the molecule agonizes target molecule/binding partner complex formation. In another embodiment, complex formation of target molecule/binding partner can be compared to complex formation of mutant target molecule/binding partner (*e.g.*, amino acid modifications in a target polypeptide). Such a comparison can be important in those cases where it is desirable to identify test molecules that modulate interactions of mutant but not non-mutated target gene products.

[0170] The assays can be conducted in a heterogeneous or homogeneous format. In heterogeneous assays, target molecule and/or the binding partner are immobilized to a solid phase, and complexes are detected on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the molecules being tested. For example, test compounds that agonize target molecule/binding partner interactions can be identified by conducting the reaction in the presence of the test molecule in a competition format. Alternatively, test molecules that agonize preformed complexes, *e.g.*, molecules with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed.

[0171] In a heterogeneous assay embodiment, the target molecule or the binding partner is anchored onto a solid surface (*e.g.*, a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored molecule can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the molecule to be anchored can be used to anchor the molecule to the solid surface. The partner of the immobilized species is exposed to the coated surface with or without the test molecule. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) such that a significant portion of any complexes formed will remain immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface is indicative of complex. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored to the surface; *e.g.*, by using a labeled antibody specific for the initially non-immobilized species. Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

[0172] In another embodiment, the reaction can be conducted in a liquid phase in the presence or absence of test molecule, where the reaction products are separated from unreacted components, and the complexes are detected (*e.g.*, using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes). Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

[0173] In an alternate embodiment, a homogeneous assay can be utilized. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared. One or both of the target molecule or binding partner is labeled, and the signal generated by the label(s) is quenched upon complex formation (*e.g.*, U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). Addition of a test molecule that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target molecule/binding partner complexes can be identified.

[0174] Candidate therapeutics for treating osteoporosis are identified from a group of test molecules that interact with a target molecule. Test molecules are normally ranked according to the degree with which they modulate (*e.g.*, agonize or antagonize) a function associated with the target molecule (*e.g.*, DNA replication and/or processing, RNA transcription and/or processing, polypeptide production and/or processing, and/or biological function/activity), and then top ranking modulators are selected. Also, pharmacogenomic information described herein can determine the rank of a modulator. The top 10% of ranked test molecules often are selected for further testing as candidate therapeutics, and sometimes the top 15%, 20%, or 25% of ranked test molecules are selected for further testing as candidate therapeutics. Candidate therapeutics typically are formulated for administration to a subject.

Therapeutic Formulations

[0175] Formulations and pharmaceutical compositions typically include in combination with a pharmaceutically acceptable carrier one or more target molecule modulators. The modulator often is a test molecule identified as having an interaction with a target molecule by a screening method described above. The modulator may be a compound, an antisense nucleic acid, a ribozyme, an antibody, or a binding partner. Also, formulations may comprise a target polypeptide or fragment thereof in combination with a pharmaceutically acceptable carrier.

[0176] As used herein, the term "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions. Pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0177] A pharmaceutical composition typically is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium

bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0178] Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0179] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0180] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile

injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0181] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[0182] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art. Molecules can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0183] In one embodiment, active molecules are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[0184] It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0185] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Molecules which exhibit high therapeutic indices are preferred. While molecules that exhibit toxic side effects may be used, care should be taken to design a delivery

system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0186] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such molecules lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any molecules used in the methods described herein, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0187] As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, sometimes about 0.01 to 25 mg/kg body weight, often about 0.1 to 20 mg/kg body weight, and more often about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, sometimes between 2 to 8 weeks, often between about 3 to 7 weeks, and more often for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

[0188] With regard to polypeptide formulations, featured herein is a method for treating osteoporosis in a subject, which comprises contacting one or more cells in the subject with a first polypeptide, where the subject comprises a second polypeptide having one or more polymorphic variations associated with low BMD, and where the first polypeptide comprises fewer polymorphic variations associated with low BMD than the second polypeptide. The first and second polypeptides are encoded by a nucleic acid which comprises a nucleotide sequence in SEQ ID NO's:1-10; a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence referenced in SEQ ID NO's:1-10; a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO's:1-10 and a nucleotide sequence 90% or more identical to a nucleotide sequence in SEQ ID NO's:1-10. The subject often is a human.

[0189] For antibodies, a dosage of 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg) is often utilized. If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is often appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life

within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (*e.g.*, into the brain). A method for lipidation of antibodies is described by Cruikshank *et al.*, *J. Acquired Immune Deficiency Syndromes* and *Human Retrovirology 14:*193 (1997).

[0190] Antibody conjugates can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide such as tumor necrosis factor, .alpha.-interferon, .beta.-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

[0191] For compounds, exemplary doses include milligram or microgram amounts of the compound per kilogram of subject or sample weight, for example, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid described herein, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

[0192] With regard to nucleic acid formulations, gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent 5,328,470) or by stereotactic injection (*see e.g.*, Chen *et al.*, (1994) *Proc. Natl. Acad. Sci. USA 91*:3054-3057). Pharmaceutical preparations of gene therapy vectors can include a gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells (*e.g.*, retroviral vectors) the pharmaceutical preparation can include one or more cells which produce the gene delivery system. Examples of gene delivery vectors are described herein.

Therapeutic Methods

[0193] A therapeutic formulation described above can be administered to a subject in need of a therapeutic for inducing a desired biological response.. Therapeutic formulations can be administered by any of the paths described herein. With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from pharmacogenomic analyses described herein.

[0194] As used herein, the term "treatment" is defined as the application or administration of a therapeutic formulation to a subject, or application or administration of a therapeutic agent to an isolated tissue or cell line from a subject with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect osteoporosis, symptoms of osteoporosis or a predisposition towards osteoporosis. A therapeutic formulation includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides. Administration of a therapeutic formulation can occur prior to the manifestation of symptoms characteristic of low BMD, such that osteoporosis is prevented or delayed in its progression. The appropriate therapeutic composition can be determined based on screening assays described herein.

[0195] As discussed, successful treatment of osteoporosis can be brought about by techniques that serve to agonize target molecule expression or function, or alternatively, antagonize target molecule expression or function. These techniques include administration of modulators that include, but are not limited to, small organic or inorganic molecules; antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, $F(ab')_2$ and Fab expression library fragments, scFV molecules, and epitope-binding fragments thereof); and peptides, phosphopeptides, or polypeptides.

[0196] Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above. It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular polypeptide, it can be preferable to co-administer normal target gene polypeptide into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

[0197] Another method by which nucleic acid molecules may be utilized in treating or preventing osteoporosis is use of aptamer molecules specific for target molecules. Aptamers are nucleic acid

molecules having a tertiary structure which permits them to specifically bind to ligands (*see, e.g.*, Osborne, *et al., Curr. Opin. Chem. Biol.1(1):* 5-9 (1997); and Patel, D. J., *Curr. Opin. Chem. Biol. Jun;1(1):* 32-46 (1997)).

[0198] Yet another method of utilizing nucleic acid molecules for osteoporosis treatment is gene therapy, which can also be referred to as allele therapy. Provided herein is a gene therapy method for treating osteoporosis in a subject, which comprises contacting one or more cells in the subject or from the subject with a nucleic acid having a first nucleotide sequence. Genomic DNA in the subject comprises a second nucleotide sequence having one or more polymorphic variations associated with low BMD (*e.g.*, the second nucleic acid has a nucleotide sequence in SEQ ID NO's:1-10). The first and second nucleotide sequences typically are substantially identical to one another, and the first nucleotide sequence comprises fewer polymorphic variations associated with low BMD than the second nucleotide sequence. The first nucleotide sequence may comprise a gene sequence that encodes a full-length polypeptide or a fragment thereof. The subject is often a human. Allele therapy methods often are utilized in conjunction with a method of first determining whether a subject has genomic DNA that includes polymorphic variants associated with low BMD.

[0199] In another allele therapy embodiment, provided herein is a method which comprises contacting one or more cells in the subject or from the subject with a polypeptide encoded by a nucleic acid having a first nucleotide sequence. Genomic DNA in the subject comprises a second nucleotide sequence having one or more polymorphic variations associated with low BMD (*e.g.*, the second nucleic acid has a nucleotide sequence in SEQ ID NO's:1-10). The first and second nucleotide sequences typically are substantially identical to one another, and the first nucleotide sequence comprises fewer polymorphic variations associated with low BMD than the second nucleotide sequence. The first nucleotide sequence may comprise a gene sequence that encodes a full-length polypeptide or a fragment thereof. The subject is often a human.

[0200] For antibody-based therapies, antibodies can be generated that are both specific for target molecules and that reduce target molecule activity. Such antibodies may be administered in instances where antagonizing a target molecule function is appropriate for the treatment of osteoporosis.

[0201] In circumstances where stimulating antibody production in an animal or a human subject by injection with a target molecule is harmful to the subject, it is possible to generate an immune response against the target molecule by use of anti-idiotypic antibodies (*see, e.g.*, Herlyn, *Ann. Med.;31(1):* 66-78 (1999); and Bhattacharya-Chatterjee & Foon, *Cancer Treat. Res.; 94:* 51-68 (1998)). Introducing an anti-idiotypic antibody to a mammal or human subject often stimulates production of anti-anti-idiotypic antibodies, which typically are specific to the target molecule. Vaccines directed to osteoporosis also may be generated in this fashion.

[0202] In instances where the target molecule is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the

antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population (*see, e.g.*, Marasco *et al., Proc. Natl. Acad. Sci. USA 90:* 7889-7893 (1993)).

[0203] Modulators can be administered to a patient at therapeutically effective doses to treat osteoporosis. A therapeutically effective dose refers to an amount of the modulator sufficient to result in amelioration of symptoms of osteoporosis. Toxicity and therapeutic efficacy of modulators can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Modulators that exhibit large therapeutic indices are preferred. While modulators that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such molecules to the site of affected tissue in order to minimize potential damage to uninfected cells, thereby reducing side effects.

[0204] Data obtained from cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods described herein, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

[0205] Another example of effective dose determination for an individual is the ability to directly assay levels of "free" and "bound" compound in the serum of the test subject. Such assays may utilize antibody mimics and/or "biosensors" that have been created through molecular imprinting techniques. Molecules that modulate target molecule activity are used as a template, or "imprinting molecule", to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated "negative image" of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell *et al., Current Opinion in Biotechnology 7:* 89-94 (1996) and in Shea, *Trends in Polymer Science 2:* 166-173 (1994). Such "imprinted" affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the

use of such matrixes in this way can be seen in Vlatakis, *et al., Nature 361:* 645-647 (1993). Through the use of isotope-labeling, the "free" concentration of compound which modulates target molecule expression or activity readily can be monitored and used in calculations of IC_{50}. Such "imprinted" affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes readily can be assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC_{50}. An example of such a "biosensor" is discussed in Kriz *et al., Analytical Chemistry 67:* 2142-2144 (1995).

[0206] The examples set forth below are intended to illustrate but not limit the invention.

Examples

[0207] In the following studies a group of subjects were selected according to specific parameters relating to low BMD. Nucleic acid samples obtained from individuals in the study group were subjected to genetic analysis, which identified associations between low BMD and certain polymorphic variants in *CETP, PROL4, GRID2, PDE4D* and *GPX3* (herein referred to as "target genes", "target nucleotides", "target polypeptides" or simply "targets"). Methods are described for producing *CETP, PROL4, GRID2, PDE4D* and *GPX3* polypeptides and polypeptide variants *in vitro* or *in vivo*. *CETP, PROL4, GRID2, PDE4D* and *GPX3* nucleic acids or polypeptides and variants thereof are utilized for screening test molecules for those that interact with *CETP, PROL4, GRID2, PDE4D* and *GPX3* molecules. Test molecules identified as being interactors with target polypeptides can be screened further as osteoporosis therapeutics.

Example 1

Samples and Pooling Strategies

Sample Selection

[0208] Blood samples were collected from individuals with low BMD, which were referred to as case samples. Also, blood samples were collected from individuals with high BMD (*i.e.*, not diagnosed with osteoporosis or any form of osteoporosis-related disease); these samples served as gender and age-matched controls. All of the samples were of Caucasian (English) descent. A database was created that listed all phenotypic trait information gathered from individuals for each case and control sample. Genomic DNA was extracted from each of the blood samples for genetic analyses.

DNA Extraction from Blood Samples

[0209] Six to ten milliliters of whole blood was transferred to a 50 ml tube containing 27 ml of red cell lysis solution (RCL). The tube was inverted until the contents were mixed. Each tube was incubated for 10 minutes at room temperature and inverted once during the incubation. The tubes were then centrifuged for 20 minutes at 3000 x g and the supernatant was carefully poured off. 100-200 µl

of residual liquid was left in the tube and was pipetted repeatedly to resuspend the pellet in the residual supernatant. White cell lysis solution (WCL) was added to the tube and pipetted repeatedly until completely mixed. While no incubation was normally required, the solution was incubated at 37°C or room temperature if cell clumps were visible after mixing until the solution was homogeneous. 2 ml of protein precipitation was added to the cell lysate. The mixtures were vortexed vigorously at high speed for 20 sec to mix the protein precipitation solution uniformly with the cell lysate, and then centrifuged for 10 minutes at 3000 x g. The supernatant containing the DNA was then poured into a clean 15 ml tube, which contained 7 ml of 100% isopropanol. The samples were mixed by inverting the tubes gently until white threads of DNA were visible. Samples were centrifuged for 3 minutes at 2000 x g and the DNA was visible as a small white pellet. The supernatant was decanted and 5 ml of 70% ethanol was added to each tube. Each tube was inverted several times to wash the DNA pellet, and then centrifuged for 1 minute at 2000 x g. The ethanol was decanted and each tube was drained on clean absorbent paper. The DNA was dried in the tube by inversion for 10 minutes, and then 1000 µl of 1X TE was added. The size of each sample was estimated, and less TE buffer was added during the following DNA hydration step if the sample was smaller. The DNA was allowed to rehydrate overnight at room temperature, and DNA samples were stored at 2-8°C.

[0210] DNA was quantified by placing samples on a hematology mixer for at least 1 hour. DNA was serially diluted (typically 1:80, 1:160, 1:320, and 1:640 dilutions) so that it would be within the measurable range of standards. 125 µl of diluted DNA was transferred to a clear U-bottom microtitre plate, and 125 µl of 1X TE buffer was transferred into each well using a multichannel pipette. The DNA and 1X TE were mixed by repeated pipetting at least 15 times, and then the plates were sealed. 50 µl of diluted DNA was added to wells A5-H12 of a black flat bottom microtitre plate. Standards were inverted six times to mix them, and then 50 µl of 1X TE buffer was pipetted into well A1, 1000 ng/ml of standard was pipetted into well A2, 500 ng/ml of standard was pipetted into well A3, and 250 ng/ml of standard was pipetted into well A4. PicoGreen (Molecular Probes, Eugene, Oregon) was thawed and freshly diluted 1:200 according to the number of plates that were being measured. PicoGreen was vortexed and then 50µl was pipetted into all wells of the black plate with the diluted DNA. DNA and PicoGreen were mixed by pipetting repeatedly at least 10 times with the multichannel pipette. The plate was placed into a Fluoroskan Ascent Machine (microplate fluorometer produced by Labsystems) and the samples were allowed to incubate for 3 minutes before the machine was run using filter pairs 485 nm excitation and 538 nm emission wavelengths. Samples having measured DNA concentrations of greater than 450 ng/µl were re-measured for conformation. Samples having measured DNA concentrations of 20 ng/µl or less were re-measured for confirmation.

Pooling Strategies

[0211] Samples were placed into one of two groups based on BMD levels. The two groups were made up of individuals with low BMD levels and individuals with high BMD levels. A select set of samples from each group were utilized to generate pools, and one pool was created for each group. Each individual sample in a pool was represented by an equal amount of genomic DNA. For example, where 25 ng of genomic DNA was utilized in each PCR reaction and there were 200 individuals in each pool, each individual would provide 125 pg of genomic DNA. Inclusion or exclusion of samples for a pool was based upon the following criteria: the sample was derived from an individual of Caucasian paternal and maternal descent; the database included relevant phenotype information for the individual; case samples were derived from individuals with low BMD; control samples were derived from individuals with normal or high BMD and no history of osteoporosis or osteoporosis-related diseases; and sufficient genomic DNA was extracted from each blood sample for all allelotyping and genotyping reactions performed during the study. Phenotype information included pre- or post-menopausal, familial predisposition, country or origin of mother and father, diagnosis with osteoporosis (date of primary diagnosis, age of individual as of primary diagnosis, osteoporosis-related fracture), biochemical measurements of markers of bone resorption (bone-specific alkaline Phosphatase, Urinary C-telopeptide of type I collagen, serum osteocalcin), current medication status (thyroid medication, hormone replacement therapy, steroid usage, bisphosphonates and cytotoxic agents for rheumatic diseases). Samples that met the inclusion criteria and did not meet the exclusion criteria were added to appropriate pools based on gender and disease status.

The selection process yielded the pools set forth in Table 2, which were used in the studies that follow. The average (mean) T-score in the High group is 1.56, and in the Low group -2.1 which means that on average individuals in the high BMD pool are 1.56 standard deviations above the average BMD in young females, whereas individuals in the low BMD pool are on average 2.1 standard deviations below the normal young value.

TABLE 2

	Female High BMD	Female Low BMD
Pool size (Number)	321	319
Mean T-score (BMD leveles adjusted for age and BMI)	1.56	-2.10
Mean Age (ex: years)	52.9	52.4

Example 2

Association of Polymorphic Variants with Low BMD

[0212] A whole-genome screen was performed to identify particular SNPs associated with low BMD. As described in Example 1, two groups of samples were utilized, which included samples from

female individuals having low BMD (osteoporosis cases), and samples from female individuals having high BMD levels (controls). The initial screen of each pool was performed in an allelotyping study, in which certain samples in each group were pooled. By pooling DNA from each group, an allele frequency for each SNP in each group was calculated. These allele frequencies were then compared to one another. Particular SNPs were considered as being associated with low BMD when allele frequency differences calculated between case and control pools were statistically significant. SNP disease association results obtained from the allelotyping study were then validated by genotyping each associated SNP across all samples from each pool. The results of the genotyping were then analyzed, allele frequencies for each group were calculated from the individual genotyping results, and a p-value was calculated to determine whether the case and control groups had statistically significantly differences in allele frequencies for a particular SNP. When the genotyping results agreed with the original allelotyping results, the SNP disease association was considered validated at the genetic level.

SNP Panel Used for Genetic Analyses

[0213] A whole-genome SNP screen began with an initial screen of approximately 25,000 SNPs over each set of disease and control samples using a pooling approach. The pools studied in the screen are described in Example 1. The SNPs analyzed in this study were part of a set of 25,488 SNPs confirmed as being statistically polymorphic as each is characterized as having a minor allele frequency of greater than 10%. The SNPs in the set reside in genes or in close proximity to genes, and many reside in gene exons. Specifically, SNPs in the set are located in exons, introns, and within 5,000 base-pairs upstream of a transcription start site of a gene. In addition, SNPs were selected according to the following criteria: they are located in ESTs; they are located in Locuslink or Ensembl genes; and they are located in Genomatix promoter predictions. SNPs in the set were also selected on the basis of even spacing across the genome, as depicted in Table 3. An additional 3088 SNPs were included with these 25,488 SNPs and these additional SNPs had been chosen on the basis of gene location, with preference to non-synonymous coding SNPs located in disease candidate genes.

TABLE 3

General Statistics		Spacing Statistics	
Total # of SNPs	25,488	Median	37,058 bp
# of Exonic SNPs	>4,335 (17%)	Minimum*	1,000 bp
# SNPs with refSNP ID	20,776 (81%)	Maximum*	3,000,000 bp
Gene Coverage	>10,000	Mean	122,412 bp
Chromosome Coverage	All	Std Deviation	373,325 bp
		*Excludes outliers	

Allelotyping and Genotyping Results

[0214] The genetic studies summarized above and described in more detail below identified allelic variants associated with low BMD. The allelic variants identified from the SNP panel described in Table 3 are summarized below in Table 4.

TABLE 4

SNP Reference	Chromosome	Chromosome Position	Position in SEQ ID Nos	Contig Identification	Contig Position	Sequence Identification	Locus	Sequence Position	Allelic Variability	Low BMD Assoc. Allele
rs1801706	16q21	57068823	50109	NT_010498	10631861	NM_000078	CETP	UTR	AG	G
rs1047699	12p13	11143886	49075	NT_009714	3758682	NM_007244	PROL4	Exon: R120Q	CT	C
rs1948017	4q22	94636365	49110	NT_016354	18829520	NM_001510	GRID2	Intron	CT	C
rs1498608	5q12	59895580	49652	NT_006713	8937426	NM_006203	PDE4D	Intron	AT	T
rs869975	5q23	150475233	50082	NT_029289	11569308	NM_002084	GPX3	Intron	AG	G

[0215] Table 4 includes information pertaining to the incident polymorphic variant associated with low BMD identified herein. Public information pertaining to the polymorphism and the genomic sequence that includes the polymorphism are indicated. The genomic sequences identified in Table 4 may be accessed at the http address www.ncbi.nih.gov/entrez/query.fcgi, for example, by using the publicly available SNP reference number (*e.g.*, rs1801706). The chromosome position refers to the position of the SNP within NCBI's Genome Build 34, which may be accessed at the following http address: www.ncbi.nlm.nih.gov/mapview/map_search.cgi?chr=hum_chr.inf&query=. The "Contig Position" provided in Table 4 corresponds to a nucleotide position set forth in the contig sequence, and designates the polymorphic site corresponding to the SNP reference number. The sequence containing the polymorphisms also may be referenced by the "Sequence Identification" set forth in Table 4. The "Sequence Identification" corresponds to cDNA sequence that encodes associated target polypeptides (*e.g.*, *CETP*) of the invention. The position of the SNP within the cDNA sequence is provided in the "Sequence Position" column of Table 4. Also, the allelic variation at the polymorphic site and the allelic variant identified as associated with low BMD is specified in Table 4. All nucleotide sequences referenced and accessed by the parameters set forth in Table 4 are incorporated herein by reference.

Assay for Verifying, Allelotyping, and Genotyping SNPs

[0216] A MassARRAY® system (Sequenom, Inc.) was utilized to perform SNP genotyping in a high-throughput fashion. This genotyping platform was complemented by a homogeneous, single-tube assay method (hME™ or homogeneous MassEXTEND™ (Sequenom, Inc.)) in which two genotyping primers anneal to and amplify a genomic target surrounding a polymorphic site of interest. A third primer (the MassEXTEND™ primer), which is complementary to the amplified target up to but not

including the polymorphism, was then enzymatically extended one or a few bases through the polymorphic site and then terminated.

[0217] For each polymorphism, SpectroDESIGNER™ software (Sequenom, Inc.) was used to generate a set of PCR primers and a MassEXTEND™ primer was used to genotype the polymorphism. Table 5 shows PCR primers and Table 6 shows extension primers used for analyzing polymorphisms. The initial PCR amplification reaction was performed in a 5 μl total volume containing 1X PCR buffer with 1.5 mM $MgCl_2$ (Qiagen), 200 μM each of dATP, dGTP, dCTP, dTTP (Gibco-BRL), 2.5 ng of genomic DNA, 0.1 units of HotStar DNA polymerase (Qiagen), and 200 nM each of forward and reverse PCR primers specific for the polymorphic region of interest.

TABLE 5: PCR Primers

Reference SNP ID	Forward PCR primer	Reverse PCR primer
rs1801706	ACGTTGGATGTTGTAGCAGAAGGCAAGCAC	ACGTTGGATGTCCATCTCCGTACTCCTAAC
rs1047699	GATTACCAGAGTGGTTGCTC	CCTGCAGGAAGCATCATCAT
rs1948017	GTTTAACAGCAACCATTGAGG	CCCCAAAGGTATGTTAAGAG
rs1498608	GAATCCCTGTTCATTCCTTG	ATACCTAGGTATAACCTCGG
rs869975	AACTCACTGGTGATCCTGCG	TGTCTCATCCACACCACTCC

[0218] Samples were incubated at 95°C for 15 minutes, followed by 45 cycles of 95°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minute, finishing with a 3 minute final extension at 72°C. Following amplification, shrimp alkaline phosphatase (SAP) (0.3 units in a 2 μl volume) (Amersham Pharmacia) was added to each reaction (total reaction volume was 7 μl) to remove any residual dNTPs that were not consumed in the PCR step. Samples were incubated for 20 minutes at 37°C, followed by 5 minutes at 85°C to denature the SAP.

[0219] Once the SAP reaction was complete, a primer extension reaction was initiated by adding a polymorphism-specific MassEXTEND™ primer cocktail to each sample. Each MassEXTEND™ cocktail included a specific combination of dideoxynucleotides (ddNTPs) and deoxynucleotides (dNTPs) used to distinguish polymorphic alleles from one another. In Table 6, ddNTPs are shown and the fourth nucleotide not shown is the dNTP.

TABLE 6: Extend Primers

Reference SNP ID	Extend Probe	Term Mix
rs1801706	CCTGGTGTCTCCTCCAGC	ACT
rs1047699	TGTCTTGCTGGTCTGTCCCTC	ACG
rs1948017	CAGCAACCATTGAGGGTGAAT	ACG
rs1498608	CCCTAAAAACTGTTCCAGGTA	CGT
rs869975	GGGCCTGAGTAGTTCCAGC	ACT

[0220] The MassEXTEND™ reaction was performed in a total volume of 9 µl, with the addition of 1X ThermoSequenase buffer, 0.576 units of ThermoSequenase (Amersham Pharmacia), 600 nM MassEXTEND™ primer, 2 mM of ddATP and/or ddCTP and/or ddGTP and/or ddTTP, and 2 mM of dATP or dCTP or dGTP or dTTP. The deoxy nucleotide (dNTP) used in the assay normally was complementary to the nucleotide at the polymorphic site in the amplicon. Samples were incubated at 94°C for 2 minutes, followed by 55 cycles of 5 seconds at 94°C, 5 seconds at 52°C, and 5 seconds at 72°C.

[0221] Following incubation, samples were desalted by adding 16 µl of water (total reaction volume was 25 µl), 3 mg of SpectroCLEAN™ sample cleaning beads (Sequenom, Inc.) and allowed to incubate for 3 minutes with rotation. Samples were then robotically dispensed using a piezoelectric dispensing device (SpectroJET™ (Sequenom, Inc.)) onto either 96-spot or 384-spot silicon chips containing a matrix that crystallized each sample (SpectroCHIP® (Sequenom, Inc.)). Subsequently, MALDI-TOF mass spectrometry (Biflex and Autoflex MALDI-TOF mass spectrometers (Bruker Daltonics) can be used) and SpectroTYPER RT™ software (Sequenom, Inc.) were used to analyze and interpret the SNP genotype for each sample.

Genetic Analysis

[0222] Variations identified in the target genes are provided in their respective genomic sequences (see SEQ ID NOs:1-4) Minor allelic frequencies for these polymorphisms was verified as being 10% or greater by determining the allelic frequencies using the extension assay described above in a group of samples isolated from 92 individuals originating from the state of Utah in the United States, Venezuela and France (Coriell cell repositories).

[0223] Genotyping results for the allelic variant set forth in Table 4 are shown for female pools in Table 7. In Table 7, "F case" and "F control" refer to female case (low BMD) and female control (high BMD) groups, and "AF" refers to allele frequency.

TABLE 7: Female Genotype Results

SNP Reference	F AF case (low)	F AF control (high)	p-value	Odds Ratio	Low BMD Associated Allele
rs1801706	A=0.15	A=0.19	0.00765	0.661	G
	G=0.85	G=0.81			
rs1047699	C=0.85	C=0.78	0.000594	1.65	C
	T=0.15	T=0.22			
rs1948017	C=0.14	C=0.10	0.0188	1.51	C
	T=0.86	T=0.90			
rs1498608	A=0.09	A=0.12	0.0347	0.672	T
	T=0.91	T=0.88			
rs869975	A=0.05	A=0.10	0.000243	0.441	G

SNP Reference	F AF case (low)	F AF control (high)	p-value	Odds Ratio	Low BMD Associated Allele
	G=0.95	G=0.90			

[0224] The single marker alleles set forth in Table 7 were considered validated, since the genotyping data were significantly associated with low BMD, and because the genotyping results agreed with the original allelotyping results. Particularly significant associations with low BMD are indicated by a calculated p-value of less than 0.05 for genotype results, which are set forth in bold text.

[0225] Odds ratio results are shown in Table 7 (and other Tables below). An odds ratio is an unbiased estimate of relative risk which can be obtained from most case-control studies. Relative risk (RR) is an estimate of the likelihood of disease in the exposed group (susceptibility allele or genotype carriers) compared to the unexposed group (not carriers). It can be calculated by the following equation:

$$RR = IA/Ia$$

*I*A is the incidence of disease in the A carriers and *I*a is the incidence of disease in the non-carriers.

RR > 1 indicates the A allele increases disease susceptibility.

RR < 1 indicates the a allele increases disease susceptibility.

For example, RR = 1.5 indicates that carriers of the A allele have 1.5 times the risk of disease than non-carriers, *i.e.*, 50% more likely to get the disease.

[0226] Case-control studies do not allow the direct estimation of *I*A and *I*a, therefore relative risk cannot be directly estimated. However, the odds ratio (OR) can be calculated using the following equation:

$$OR = (nDAnda)/(ndAnDa) = pDA(1 - pdA)/pdA(1 - pDA), \text{ or}$$

OR = ((case f) / (1- case f)) / ((control f) / (1-control f)), where f = susceptibility allele frequency.

[0227] An odds ratio can be interpreted in the same way a relative risk is interpreted and can be directly estimated using the data from case-control studies, *i.e.*, case and control allele frequencies. The higher the odds ratio value, the larger the effect that particular allele has on the development of low BMD. Possessing an allele associated with a relatively high odds ratio translates to having a higher risk of developing or having low BMD.

Example 3

CETP Region Proximal SNPs

[0228] It has been discovered that a polymorphic variation (rs1801706) in a gene encoding *CETP* is associated with the occurrence of low BMD (see Examples 1 and 2). Ninety-one additional allelic variants proximal to rs1801706 were identified and subsequently allelotyped in low BMD case and high BMD control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 8. The chromosome positions provided in column four of Table 8 are based on Genome

"Build 34" of NCBI's GenBank. The "genome letter" corresponds to the particular allele that appears in NCBI's build 34 genomic sequence of the region (chromosome 16: positions 56743155-56840953), and the "deduced iupac" corresponds to the single letter IUPAC code for the *CTEP* polymorphic variants as they appear in SEQ ID NO:1. Also, the "genome letter" may differ from the alleles (A1/A2) provided in Table 8 (and in subsequent Tables that provide the same information) if the genome letter is on one strand and the alleles are on the complementary strand, thus having different strand orientations (*i.e.*, reverse vs forward).

TABLE 8

dbSNP	Position in SEQ ID NO:1	Chromo -some	Chromosome Position	Alleles (A1/A2)	Genome letter	Deduced iupac
7500979	205	16	56743155	g/a	g	R
2217332	1595	16	56744545	g/a	g	R
8044804	2650	16	56745600	c/t	a	R
2270835	5496	16	56748446	c/t	t	Y
2133783	5782	16	56748732	g/a	a	R
247609	5908	16	56748858	c/t	g	R
952440	7552	16	56750502	g/a	a	R
881598	9191	16	56752141	t/c	g	R
2291955	10127	16	56753077	g/a	t	Y
2518054	10345	16	56753295	g/a	g	R
866038	10399	16	56753349	t/c	c	Y
1436425	12028	16	56754978	g/a	a	R
173537	13355	16	56756305	a/g	t	Y
247611	13687	16	56756637	a/g	g	R
166017	14328	16	56757278	t/c	c	Y
173538	14746	16	56757696	c/t	t	Y
193694	14996	16	56757946	t/c	c	Y
7205692	19361	16	56762311	g/a	a	R
8048746	21775	16	56764725	g/a	a	R
247618	23250	16	56766200	g/a	g	R
183130	23810	16	56766760	c/t	c	Y
6499863	24464	16	56767414	g/a	g	R
4783961	27341	16	56770291	g/a	g	R
3816117	28605	16	56771555	c/t	t	Y
711752	28658	16	56771608	g/a	g	R
708272	28735	16	56771685	c/t	g	R
1864163	29680	16	56772630	g/a	g	R
4369653	29998	16	56772948	g/a	c	Y
1864165	32521	16	56775471	c/t	c	Y
891141	36170	16	56779120	t/g	g	K
891143	36427	16	56779377	c/t	t	Y
7205804	37336	16	56780286	g/a	g	R
5885	37718	16	56780668	c/t	c	Y
1532625	37748	16	56780698	a/g	c	Y
1532624	37926	16	56780876	t/g	c	M
289712	38752	16	56781702	g/a	c	Y
7499892	39037	16	56781987	c/t	c	Y

dbSNP	Position in SEQ ID NO:1	Chromo -some	Chromosome Position	Alleles (A1/A2)	Genome letter	Deduced iupac
5883	39800	16	56782750	c/t	c	Y
289714	39898	16	56782848	c/t	g	R
158480	40674	16	56783624	c/t	g	R
289717	41835	16	56784785	c/t	g	R
4344729	42325	16	56785275	c/t	g	R
289718	42379	16	56785329	a/g	c	Y
289719	42388	16	56785338	g/a	t	Y
2033254	42432	16	56785382	c/t	t	Y
4784744	43632	16	56786582	g/a	g	R
291044	43899	16	56786849	c/t	g	R
8053613	44273	16	56787223	c/t	c	Y
5881	44459	16	56787409	a/g	g	R
5880	47538	16	56790488	c/g	g	S
7198026	47692	16	56790642	c/t	t	Y
5882	48539	16	56791489	g/a	g	R
8045701	48749	16	56791699	c/t	t	Y
289741	49921	16	56792871	a/g	g	R
1801706	50109	16	56793059	a/g	g	R
289742	50209	16	56793159	c/g	c	S
289743	50243	16	56793193	c/t	g	R
289746	52652	16	56795602	g/a	c	Y
172337	55195	16	56798145	c/t	t	Y
289747	56385	16	56799335	a/g	c	Y
1566439	57109	16	56800059	a/g	t	Y
7205459	57618	16	56800568	c/t	t	Y
289749	58741	16	56801691	t/c	g	R
289751	59222	16	56802172	t/c	g	R
8059220	60771	16	56803721	c/t	c	Y
8058353	60962	16	56803912	g/a	g	R
289735	62009	16	56804959	a/g	c	Y
289737	64589	16	56807539	t/g	a	M
291042	66054	16	56809004	g/a	a	R
1875236	66143	16	56809093	c/t	g	R
821466	67822	16	56810772	a/g	t	Y
821465	68805	16	56811755	c/g	c	S
4275846	70075	16	56813025	c/t	g	R
289707	70350	16	56813300	a/g	t	Y
821463	71214	16	56814164	g/a	t	Y
289706	79549	16	56822499	c/t	c	Y
1167741	82760	16	56825710	c/t	c	Y
2052880	86463	16	56829413	a/c	g	K
1167742	86533	16	56829483	c/g	c	S
1183256	87019	16	56829969	a/g	a	R
1651665	88910	16	56831860	a/c	a	M
1651666	88955	16	56831905	c/t	c	Y
4784751	89021	16	56831971	c/t	c	Y
1651667	89056	16	56832006	a/g	a	R
8052091	89863	16	56832813	g/a	g	R
1684574	89879	16	56832829	t/c	t	Y

dbSNP	Position in SEQ ID NO:1	Chromo -some	Chromosome Position	Alleles (A1/A2)	Genome letter	Deduced iupac
1684575	90066	16	56833016	t/g	g	K
1672865	90101	16	56833051	a/g	a	R
821470	91029	16	56833979	g/a	a	R
1549669	91434	16	56834384	t/g	a	M
291040	93636	16	56836586	t/c	t	Y
289754	98003	16	56840953	c/t	c	Y

Assay for Verifying and Allelotyping SNPs

[0229] The methods used to verify and allelotype the proximal SNPs of Table 8 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 9 and Table 10, respectively.

TABLE 9

dbSNP rs#	Forward PCR primer	Reverse PCR primer
7500979	TTGTATATGTAGGGTCCTGC	AAAATAATTCAAAACCACTG
2217332	ATCCAACAACAGCTTCCCAG	GGTGAAATGCTGACCTGTGT
8044804	ATAAATACAACCCAGCCCAC	CATTTCATTTCCCTGCACTG
2270835	CATTTACCATGTAACTGCCG	GGGTTGAGTATGAACAAATG
2133783	ACACTCATCCCTCCATTCTG	TCCCGGGAATTGAAGGGAAT
247609	GTCACTAAACTATGTATCAG	GGGTAGAGTGTAAATGACAG
952440	AGTCAGTGCCTGACTTTACC	CATACTGAGGCATGGAACAG
881598	ACTGCACTCCAGCCTGGGTGA	TGCTCCTGGCAGCAAATATC
2291955	GCTAGACATGTTTTAGCAGG	CCCCTTAGTTTAAAGTAAAGC
2518054	CTGATCATTCTTACCGGCAC	CTAGACAGTCAACAACTGAG
866038	CAAGGAACAAAGCAAGACCC	GTGCCGGTAAGAATGATCAG
1436425	GTATTCAGAGTGGTGTGTGG	GGGACATGGCAGAAATTCAG
173537	TCCCTGTCCTTGAACTCATC	CCTCTTGGGTCTTGTAGTCG
247611	AGGGTCCTCCATGATTGGAG	TTTCTGGACCTGACTGGGTG
166017	TAGATGGGCTGTTTCTACTG	AGGGAAGATTCCAGTAAAGC
173538	TCCCAAAGTGCTGGGATTAC	GGGTTTCTCAAAGGGCTAAG
193694	TCCTTCCGTGATCTCACAAC	CACACCAAAAGAACTGCATG
7205692	TCTTAGCCTGGGACTTTCTG	AAAAGCAGCTGTGACCTAAG
8048746	CAAGTTCTTCCTCCATCCAC	TTCCTCTCTCTGGGCTTATC
247618	TTTTCCCCCTTTTTGGGGC	TGGGTTGTCACAGCAAGGTGG
183130	ATAAACGGGAAAGAAGAGAC	AGGGTGGAGAATCTACAGAA
6499863	GTCTTGACAGGTTTGAGGAG	CATCAGACAGATCCCAACAG
4783961	GAAACATGAGTCGGGATGGC	AGCTTTGGTATTGGAGCAGG
3816117	ACTAGCCCAGAGAGAGGAGT	AAGAACTTGACCTTGAAGGC
711752	CACAAATCCCTATACCTGGC	TCGCCTTCAAGGTCAAGTTC
708272	AACCTGGCTCAGATCTGAAC	GCCAGGTATAGGGATTTGTG
1864163	TTAGAGGGGCTGTTGGAGAG	AGAGCCTGACACCTTCCCTA
4369853	AGTATCTTGACTTTATTTGG	GAAAAAAATATATGATAAAGG
1864165	TAAAAGGCTAGAAGTCCACC	AATTAATTCTCCCCTATAGC
891141	AGGCCCAGCCTGGGAAGTTT	TATCAGATGGTATCCACATG
891143	TCGTGCCCATCCTGTTAGTG	CACAAGCATGCCCTGTGTGG

dbSNP rs#	Forward PCR primer	Reverse PCR primer
7205804	AGGCAGCAAGCACCACAATG	ATGAACGGTGCCTGGTACAC
5885	GCAAAGAGATCAACGTCATC	ATGCAGACAGAAACGCACTCA
1532625	ACTGCTGTCTTCTGAGGCAT	CATCATGGCCGATTTTGTCC
1532624	TCTATAGACTTGCCCAACGC	TACTTTGGCAAATCTCTGCC
289712	ATTCGGCTTCTGTCATCCTC	GGGTCCAAAGCTTTTGTCAG
7499892	TGGCTGACTGGCCTGACCAC	CCCTCCATTCTGTACCACTTA
5883	TACTTCTGGTTCTCTGAGCG	TTGAACTCGTCTCCCATCAG
289714	ACACACACATACCACATGCC	TGATGGGAGACGAGTTCAAG
158480	TGAGTAGTTGGGACTATAGG	ACCCCTGTCTCTACAAAAAT
289717	CTCTGAGCCCAGAGTTGATC	CATTCCCTGCTCCATTTCCC
4344729	GCCTGAGTTCAGAAGGGAAG	GGCCGTTCTCCTGTTCTAAC
289718	GCCTGCCCAATATTGTGAGT	TCTTCCCTTCTGAACTCAGG
289719	TCTTCCCTTCTGAACTCAGG	GCCTGCCCAATATTGTGAGT
2033254	CACAACTCACAATATTGGGC	CAAAGGAACAGGACTCAGAC
4784744	ATCACATGCCCCAAGAAACC	CCGGCCCTTTCTTTCTTTTG
291044	AGGGCATCCCAGAACAGAAC	TGACTAGGTCAGGTCCCCTC
8053613	GAGTTCAGGGTAGGAATAGC	GCTGTGCAAATTAGGACTCT
5881	GACCCCTGTCTTCCACAGGT	ATCTTGGGCATCTTGAGGCA
5880	TTTCTCTCCCCAGGATATCG	CCAAGAGGCTTAAGAAGAGC
7198026	TGTTGGTGGGGAAATGTGGC	ACGAAGATTCTATCTAGGCA
5882	TCCAGGGAGGACTCACCATG	TGACTGCAGGAAGCTCTGG
8045701	TTGATACTTAGCGGTCCTGG	TAATATTCTGCAGGTAAACC
289741	TCTACCAGCTTGGCTCCCTC	AAGGGAGGGGCAGTAGGAGA
1801706	GTAGCAGAAGGCAAGCAC	AGGGAGGAGTTGGGAGCC
289742	ACTGGTGAGACAATCCCTTC	CCACTGGCATTAAAGTGCTG
289743	TGAAAAGAGGTGGACGGCAC	AGTCCTTCTTCTGTGGCTGG
289746	GAGGCTTACCAAAATGGGAC	AGAGCTTCTAGGCTTGGATG
172337	AGCTGGACTTTGAGGATGGC	AAGGAGAGGAGGGACTGGAG
289747	GTTGTTTAGGCCAAAAAGTC	CAATTACGGAAGTTACACTG
1566439	AAGCCCATGGCTTTTCAAGG	AGATCCTGGAGCCTCATTGT
7205459	GCAGAGGGGAAAGATCTTGG	AAGAGGGTATGTGTGTTGG
289749	TGAACTAAGGACCAGGCAAG	GCTCACATCCTTAGATTGCC
289751	AAATGATATGGAATTATGCG	TATCTTTCCAAATGTTTTAC
8059220	ACCTGCCATCATGAGTGCAT	TAGAGCAGGGCCTGGTGTGT
8058353	CCCTACAAGAAGGCTACATC	AGTGACTGCAGTGAAGAAGG
289735	CAAGGCATGCATGCTCCTTC	ATCGCCAGACCCTCAAGAAC
289737	GCAAGGAAGACTGATTCGTG	AGCCTCAAGTCACTGATGTC
291042	TGTCTCCCCATAACCAACAC	AAGAAACAGCTCCCAGTCTG
1875236	GAGCTGAGTGATTCTTGTCC	AATATGGTCAACTTGGTGGC
821466	AAGGAAGGAAGTGAGGGATG	GGAAAGCAAAGGAATGGCAG
821465	CACTGTTTACAATAGCCAAG	TGGAATTACAGGCGTGAGCT
4275846	GCAGCTGAGCAAAGATATGG	TGGACCTCTTCTATCATCAC
289707	CTCCTTCCTTCTGCTATCTC	ACCAAGATTCAGGCACTGAG
821463	CGTCTGCTGGGACACTGAAA	GCACCTGGAAGATTTATGGG
289706	TTAAGTCCTTTACCAAAGGG	TGAATGTGGCTTTACCAAGG
1167741	GCCCTATTTACGTGGATTTG	GCTCTGATTGTGTCTCTGTG
2052880	CATCAAGACTCCATGGAGAG	AGTCAAGAACCAGTCCCTAC
1167742	CCCTAGAAACTCCCTTATCC	TGAGTGGGGTACAGATGAAC
1183256	AGCTCAACTCCCCCCAAGTT	GAAGATAGGTGAGTTGAGGG

dbSNP rs#	Forward PCR primer	Reverse PCR primer
1651665	AAGTGAGAACAGGCTTCCTG	TCAAGGCTTTAGCTTGTCCC
1651666	TCTAACCTGGAACCCCTCAG	ATGAAGCCTGACACTTTGGG
4784751	CAAAGTGTCAGGCTTCATGG	AGCTGTGGGTGAGTGTCAAG
1651667	TCCCGACTTGTACACCTTGG	AGAAAATCAGCCAAAGGCTC
8052091	AGTCTTGCAGTCCAGGATG	TTCCCTGATGGACAGATGGC
1684574	TCTTGCAGTCCAGGATGCAG	GGCCATTTAGTGCTTGGAGC
1684575	TTTCCAGTCCCTGCATGTG	GAGGGGTTACCCAGAACCTT
1672865	AAAGGTTCTGGGTAACCCCT	CAGGCCTGAAAAAGCAAAGG
821470	CCTCTTTCTGTAATATCTGG	CTGGGCATTGCAGAACTGAA
1549669	CTGCAATAGTACACGTGGTG	AGATGTTGCAAAGGAGGTGC
291040	TACACTTGCCCAAAGTCCAC	TCTCTCTGTCAATCATGGGC
289754	ACTGCTTAGGTTGGCAAAGG	ATGCTTCCTTCCACAGGGAC

TABLE 10

dbSNP rs#	Extend Primer	Term Mix
7500979	TCAACCAATAGAAAAGGC	ACG
2217332	AACCTCTGGTCCTCTGGA	ACG
8044804	TAAGCCTTGGTATGATAC	ACG
2270835	GCCGTAAATTCCATTCTTC	ACG
2133783	CTGAACTTTACAGGTAACA	ACG
247609	AAACTATGTATCAGACAAAAGCAC	ACG
952440	CCTGGAAGGCAGCTGTGG	ACG
881598	CAGCCTGGGTGACAGAGC	ACT
2291955	GACTTTTCTAGGAAAGACTTA	ACG
2518054	TACCGGCACAAACAGTC	ACG
866038	CCCATCTCAAAAACAAAAAC	ACT
1436425	GGTGTGTGGCCCATGAT	ACG
173537	CATCACACCTGTCTGCCATC	ACT
247611	CTCCATGATTGGAGACTGACA	ACT
186017	CTACTGGCAGGGAACAT	ACT
173538	TTACAGGCGTAAGCCAC	ACG
193694	CTTAAAATCTACTCCCATACAT	ACT
7205692	CAAAAGAGTTAGGGGAG	ACG
8048746	CATGCCAAAATCTCGCC	ACG
247618	TTGGGGCCCCATGTAAA	ACG
183130	CTTCTGTGCAGGAGAAT	ACG
6499863	TTGAGGAGCAGTGGTCA	ACG
4783961	GGGTCCTGCCCTAGTCC	ACG
3816117	GAGGGAGATGGGCTGAG	ACG
711752	CAATGCAGCTAGGACCTTCT	ACG
708272	CTGGCTCAGATCTGAACCCTAACT	ACG
1864163	GGCTGTTGGAGAGGTTGATA	ACG
4369653	CAGGTGCTTTTACAAACAA	ACG
1864165	GTCCACCATGGCCCTCC	ACG
891141	CTGGGAAGTTTGCAGGG	ACT
891143	AGTGTGTCCACGGCTCC	ACG

dbSNP rs#	Extend Primer	Term Mix
7205804	CCTGTGCATCCATGGAG	ACG
5885	CATCTCTAACATCATGGC	ACG
1532625	TGAGGCATGCAGACAGAAAC	ACT
1532624	CAACGCCACACAGCTTGTGA	ACT
289712	CAGTGGATTGTGGCCCCC	ACG
7499892	AGCCCGTTGGCCTGAAC	ACG
5883	GTTCTCTGAGCGAGTCTT	ACG
289714	CATACCACATGCCATCTGGAT	ACG
158480	GGGACTATAGGTATGCAC	ACG
289717	TTCCAGCCCCCTACAAGTC	ACG
4344729	CAGAAGGGAAGAGGGAC	ACG
289718	TATGTGCAAGGAGAGAG	ACT
289719	CTGTGATGCCTCTCTCC	ACG
2033254	GGCAGGCCCCTGACCGGC	ACG
4784744	CCAAGAAACCACTGAAC	ACG
291044	AGTATTTAAAGGAGAGACACAC	ACG
8053613	GATGAGAAACTGAGGCC	ACG
5881	TTCCACAGGTTGTCGGC	ACT
5880	ATATCGTGACTACCGTCCAG	ACT
7198026	GAAATGTGGCCCCTTTC	ACG
5882	CAGAGCAGCTCCGAGTCC	ACT
8045701	CCTCTCCCTGCTGGTGG	ACG
289741	TGGGAGTCAGCCCAGCTC	ACT
1801706	CCTGGTGTCTCCTCCAGC	ACT
289742	GCCACAGAAGAAGGACTCC	ACT
289743	TGAGACAATCCCTTCCCCC	ACG
289746	TACCAAAATGGGACTGACCTC	ACG
172337	TTTGAGGATGGCCCCTGAC	ACG
289747	TAGGCCAAAAAGTCTAAATTGC	ACT
1566439	CCTTAGAGAAACGGAAGGTG	ACT
7205459	ACTTTATTCCTGGATCAGA	ACG
289749	CAGGCAAGCTAATGCAA	ACT
289751	TGGAATTATGCGTTAAAGG	ACT
8059220	TGCATTCAGCTTCTCCA	ACG
8058353	CTACATCTGCCTCTCTATC	ACG
289735	GAGGGACAGAAGGGACC	ACT
289737	CTTACTTGTGTGGTTTAGAAT	ACT
291042	CCCATAACCAACACTCAGCT	ACG
1875236	GATTCTTGTCCTCTGAGAGC	ACG
821466	GAGGACAGGAGTGGAGCC	ACT
821465	ACAGAGAAAATGTGGCA	ACT
4275646	GATATGGTTTCAAAAGCCT	ACG
289707	TGCCATTCTCTAGGCCCGTC	ACT
821463	TGGGACACTGAAACCAGGG	ACG
289706	ACCAAAGGGATTGACTT	ACG
1167741	GAACTCTGAGTTACATTCC	ACG
2052880	CTTCCCTGCCTATTTTATGTC	ACT
1167742	CTTATCCCCCAACTCACCTT	ACT
1183256	TCATCTGCCCCCAGTTT	ACT

dbSNP rs#	Extend Primer	Term Mix
1651665	AGGGGTTCCAGGTTAGACCCTT	ACT
1651666	AGGAAGCCTGTTCTCACTTTC	ACG
4784751	TCATGGGGAAGCCCTAA	ACG
1651667	CTTGTACACCTTGGACTTGAC	ACT
8052091	CAACAGCCAGCAGCCCC	ACG
1684574	GATGCAGGAAGCAGGGCC	ACT
1684575	CACCTGTCTGTGGACTGGA	ACT
1672865	GGGTAACCCCTCAGCCCAG	ACT
821470	TCTGGATCCCCAGTGCC	ACG
1549669	TAGTACACGTGGTGTAAGGG	ACT
291040	CCAAAGTCCACCAGCCTCT	ACT
289754	CAAAGGATTTTAACTTCCTCTAG	ACG

Genetic Analysis

[0230] Allelotyping results are shown for female cases and controls in Table 11. The allele frequency for the A2 allele is noted in the fifth and sixth columns for control pools and case pools, respectively, where "AF" is allele frequency. Some SNPs do not have an allele frequency disclosed because of failed assays.

TABLE 11

dbSNP rs#	Position in SEQ ID NO: 1	Chromo-some Position	A1/A2 Allele	High AF	Low AF	p-Value	OR	Low BMD Associated Allele
7500979	205	56743155	G/A	G=0.71	G=0.72	0.6029	1.07	G
				A=0.29	A=0.28			
2217332	1595	56744545	G/A	G=0.85	G=0.84	0.5390	0.90	A
				A=0.15	A=0.16			
8044804	2650	56745600	C/T	C=0.47	C=			
				T=0.53	T=			
2270835	5496	56748446	C/T	C=0.00	C=0.00			
				T=1.00	T=1.00			
2133783	5782	56748732	G/A	G=0.41	G=0.44	0.3412	1.14	G
				A=0.59	A=0.56			
247609	5908	56748858	C/T	C=0.70	C=0.70	0.8844	1.02	C
				T=0.30	T=0.30			
952440	7552	56750502	G/A	G=0.31	G=0.30	0.8410	0.97	A
				A=0.69	A=0.70			
881598	9191	56752141	T/C	T=0.23	T=0.25	0.4703	1.11	T
				C=0.77	C=0.75			
2291955	10127	56753077	G/A	G=0.00	G=0.00			
				A=1.00	A=1.00			
2518054	10345	56753295	G/A	G=0.87	G=0.91	0.0835	1.47	G
				A=0.13	A=0.09			
866038	10399	56753349	T/C	T=0.49	T=0.51	0.5835	1.07	T
				C=0.51	C=0.49			
1436425	12028	56754978	G/A	G=0.39	G=0.38	0.7660	0.96	A

dbSNP rs#	Position in SEQ ID NO: 1	Chromo-some Position	A1/A2 Allele	High AF	Low AF	p-Value	OR	Low BMD Associated Allele
				A=0.61	A=0.62			
173537	13355	56756305	A/G	A=0.80	A=0.81	0.8011	1.04	A
				G=0.20	G=0.19			
247611	13687	56756637	A/G	A=0.38	A=0.39	0.7529	1.04	A
				G=0.62	G=0.61			
166017	14328	56757278	T/C	T=0.29	T=0.43	0.00001	1.80	T
				C=0.71	C=0.57			
173538	14746	56757696	C/T	C=0.68	C=0.67	0.5702	0.93	T
				T=0.32	T=0.33			
193694	14996	56757946	T/C	T=0.14	T=0.24	0.0015	2.02	T
				C=0.86	C=0.76			
7205692	19361	56762311	G/A	G=0.01	G=0.01			
				A=0.99	A=0.99			
8048746	21775	56764725	G/A	G=0.00	G=0.00			
				A=1.00	A=1.00			
247618	23250	56766200	G/A	G=0.28	G=0.29	0.5339	1.08	G
				A=0.72	A=0.71			
183130	23810	56766760	C/T	C=0.64	C=0.59	0.0720	0.80	T
				T=0.36	T=0.41			
6499863	24464	56767414	G/A	G=0.83	G=0.84	0.5810	1.09	G
				A=0.17	A=0.16			
4783961	27341	56770291	G/A	G=0.48	G=0.46	0.3803	0.90	A
				A=0.52	A=0.54			
3816117	28605	56771555	C/T	C=0.56	C=0.55	0.8019	0.97	T
				T=0.44	T=0.45			
711752	28658	56771608	G/A	G=0.59	G=0.58	0.6887	0.95	A
				A=0.41	A=0.42			
708272	28735	56771685	C/T	C=0.62	C=0.62	0.9629	1.01	C
				T=0.38	T=0.38			
1864163	29680	56772630	G/A	G=0.74	G=0.74	0.9938	1.00	G
				A=0.26	A=0.26			
4369653	29998	56772948	G/A	G=0.37	G=0.37	0.8163	0.97	G
				A=0.63	A=0.63			
1864165	32521	56775471	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
891141	36170	56779120	T/G	T=1.00	T=1.00			
				G=0.00	G=0.00			
891143	36427	56779377	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
7205804	37336	56780286	G/A	G=0.72	G=0.78	0.0212	1.41	G
				A=0.28	A=0.22			
5885	37718	56780668	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
1532625	37748	56780698	A/G	A=0.60	A=0.61	0.6329	1.06	A
				G=0.40	G=0.39			
1532624	37926	56780876	T/G	T=0.64	T=0.64	0.9807	1.00	T
				G=0.36	G=0.36			
289712	38752	56781702	G/A	G=0.66	G=0.67	0.6644	1.06	G
				A=0.34	A=0.33			

dbSNP rs#	Position in SEQ ID NO: 1	Chromo-some Position	A1/A2 Allele	High AF	Low AF	p-Value	OR	Low BMD Associated Allele
7499892	39037	56781987	C/T	C=0.88 T=0.12	C=0.88 T=0.12	0.9632	1.01	C
5883	39800	56782750	C/T	C=0.95 T=0.05	C=0.96 T=0.04	0.8994	1.04	C
289714	39898	56782848	C/T	C=0.19 T=0.81	C=0.23 T=0.77	0.0972	1.28	C
158480	40674	56783624	C/T	C=0.59 T=0.41	C=0.56 T=0.44	0.3315	0.86	T
289717	41835	56784785	C/T	C=0.67 T=0.33	C=0.72 T=0.28	0.1148	1.25	C
4344729	42325	56785275	C/T	C=1.00 T=0.00	C=1.00 T=0.00			
289718	42379	56785329	A/G	A= G=	A=0.20 G=0.80			
289719	42388	56785338	G/A	G= A=	G=0.39 A=0.61			
2033254	42432	56785382	C/T	C=0.34 T=0.66	C=0.38 T=0.62	0.1947	1.17	C
4784744	43632	56786582	G/A	G=0.63 A=0.37	G=0.64 A=0.36	0.5113	1.08	G
291044	43899	56786849	C/T	C=0.65 T=0.35	C=0.65 T=0.35	0.9666	1.01	C
8053613	44273	56787223	C/T	C=1.00 T=0.00	C=1.00 T=0.00			
5881	44459	56787409	A/G	A=0.00 G=1.00	A=0.00 G=1.00			
5880	47538	56790488	C/G	C=0.09 G=0.91	C=0.09 G=0.91	0.9339	1.02	C
7198026	47692	56790642	C/T	C=0.00 T=1.00	C=0.00 T=1.00			C
5882	48539	56791489	A/G	A=0.76 G=0.24	A=0.80 G=0.20	0.0958	1.27	A
8045701	48749	56791699	C/T	C=0.00 T=1.00	C=0.00 T=1.00			
289741	49921	56792871	A/G	A=0.85 G=0.15	A=0.86 G=0.14	0.5974	1.09	A
1801706	50109	56793059	A/G	A=0.31 G=0.69	A=0.23 G=0.77	0.0025	0.67	G
289742	50209	56793159	C/G	C=0.91 G=0.09	C=0.92 G=0.08	0.6298	1.12	C
289743	50243	56793193	C/T	C=0.27 T=0.73	C=0.24 T=0.76	0.1869	0.84	T
289746	52652	56795602	G/A	G=0.72 A=0.28	G=0.72 A=0.28	0.8916	0.98	G
172337	55195	56798145	C/T	C=0.94 T=0.06	C=0.91 T=0.09	0.0886	0.67	T
289747	56385	56799335	A/G	A=0.49 G=0.51	A=0.54 G=0.46	0.0981	1.22	A
1566439	57109	56800059	A/G	A=0.66	A=0.65	0.8578	0.98	G

dbSNP rs#	Position in SEQ ID NO: 1	Chromo-some Position	A1/A2 Allele	High AF	Low AF	p-Value	OR	Low BMD Associated Allele
				G=0.34	G=0.35			
7205459	57618	56800568	C/T	C=0.16	C=0.10	0.0035	0.59	T
				T=0.84	T=0.90			
289749	58741	56801691	T/C	T=	T=0.42			
				C=	C=0.58			
289751	59222	56802172	T/C	T=0.98	T=0.94			
				C=0.02	C=0.06			
8059220	60771	56803721	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
8058353	60962	56803912	G/A	G=0.98	G=0.95			
				A=0.02	A=0.05			
289735	62009	56804959	A/G	A=	A=0.39			
				G=	G=0.61			
289737	64589	56807539	T/G	T=	T=0.28			
				G=	G=0.72			
291042	66054	56809004	G/A	G=0.80	G=0.82	0.4548	1.12	G
				A=0.20	A=0.18			
1875236	66143	56809093	C/T	C=0.91	C=0.90	0.3106	0.80	T
				T=0.09	T=0.10			
821466	67822	56810772	A/G	A=0.77	A=0.76	0.6743	0.94	G
				G=0.23	G=0.24			
821465	68805	56811755	C/G	C=0.93	C=0.87	0.0041	0.54	G
				G=0.07	G=0.13			
4275846	70075	56813025	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
289707	70350	56813300	A/G	A=0.44	A=0.46	0.6117	1.07	A
				G=0.56	G=0.54			
821463	71214	56814164	G/A	G=0.38	G=0.41	0.4281	1.10	G
				A=0.62	A=0.59			
289706	79549	56822499	C/T	C=0.00	C=0.00			
				T=1.00	T=1.00			
1167741	82760	56825710	C/T	C=0.45	C=0.46	0.7635	1.04	C
				T=0.55	T=0.54			
2052880	86463	56829413	A/C	A=0.67	A=0.68	0.8378	1.03	A
				C=0.33	C=0.32			
1167742	86533	56829483	C/G	C=0.70	C=0.69	0.7322	0.96	G
				G=0.30	G=0.31			
1183256	87019	56829969	A/G	A=1.00	A=1.00			
				G=0.00	G=0.00			
1651665	88910	56831860	A/C	A=0.30	A=0.35	0.1423	1.24	A
				C=0.70	C=0.65			
1651666	88955	56831905	C/T	C=0.71	C=0.67	0.1294	0.81	T
				T=0.29	T=0.33			
4784751	89021	56831971	C/T	C=0.70	C=0.73	0.1971	1.18	C
				T=0.30	T=0.27			
1651667	89056	56832006	A/G	A=0.44	A=0.47	0.2902	1.13	A
				G=0.56	G=0.53			
8052091	89863	56832813	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	High AF	Low AF	p-Value	OR	Low BMD Associated Allele
1684574	89879	56832829	T/C	T=0.01	T=			
				C=0.99	C=			
1684575	90066	56833016	T/G	T=0.65	T=0.67	0.3473	1.12	T
				G=0.35	G=0.33			
1672865	90101	56833051	A/G	A=0.49	A=0.50	0.6959	1.05	A
				G=0.51	G=0.50			
821470	91029	56833979	G/A	G=0.37	G=0.38	0.8340	1.03	G
				A=0.63	A=0.62			
1549669	91434	56834384	T/G	T=0.51	T=0.50	0.9075	0.99	G
				G=0.49	G=0.50			
291040	93636	56836586	T/C	T=0.76	T=0.74	0.4056	0.89	C
				C=0.24	C=0.26			
289754	98003	56840953	C/T	C=0.70	C=0.69	0.6508	0.94	T
				T=0.30	T=0.31			

[0231] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1 can be determined by consulting Table 11. For example, the left-most X on the left graph is at position 56743155. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0232] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0233] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken

horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is place at the 3' end of each gene to show the direction of transcription.

Example 4

PROL4 Proximal SNPs

[0234] It has been discovered that a polymorphic variation (rs1047699) in a gene encoding *PROL4* is associated with the occurrence of low BMD (see Examples 1 and 2). One hundred twenty-five additional allelic variants proximal to rs1047699 were identified and subsequently allelotyped in low BMD case and high BMD control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 12. The chromosome position provided in column four of Table 12 is based on Genome "Build 34" of NCBI's GenBank.

TABLE 12

dbSNP	Position in SEQ ID NO:2	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
523051	229	12	10842129	t/c	g	R
693620	368	12	10842268	t/c	a	R
2588349	583	12	10842483	g/a	g	R
2588350	2424	12	10844324	t/c	t	Y
619381	3625	12	10845525	c/t	c	Y
3759252	3709	12	10845609	c/a	g	K
3759251	3750	12	10845650	t/a	t	W
2418107	4724	12	10846624	g/c	g	S
7303054	4781	12	10846681	t/c	c	Y
1838345	6161	12	10848061	a/g	g	R
620878	7097	12	10848997	t/g	g	K
2537817	8025	12	10849925	t/c	t	Y
1548803	8398	12	10850298	c/t	c	Y
667123	10144	12	10852044	g/a	a	R
1838346	10384	12	10852284	a/g	g	R
2159903	11116	12	10853016	a/g	g	R
3944035	11132	12	10853032	a/g	g	R
3741845	11482	12	10853382	t/c	a	R
2110096	14544	12	10856444	c/t	c	Y
759055	15688	12	10857588	t/a	t	W
589377	17311	12	10859211	g/c	g	S
7960194	17831	12	10859731	g/t	t	K
7978242	20012	12	10861912	a/g	g	R
801051	21997	12	10863897	g/a	g	R
4262797	22861	12	10864761	a/g	a	R
2215714	23470	12	10865370	a/g	g	R
1373434	23515	12	10865415	g/a	g	R
2215715	23863	12	10865763	g/a	g	R
612456	24108	12	10866008	c/a	c	M
612808	24138	12	10866038	t/c	c	Y
689118	26469	12	10868369	t/c	t	Y

dbSNP	Position in SEQ ID NO:2	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_Iupac
597468	27769	12	10869669	a/g	g	R
592884	29683	12	10871583	t/c	t	Y
640372	30491	12	10872391	a/c	c	M
7966559	30745	12	10872645	a/t	a	W
654834	31429	12	10873329	t/c	t	Y
4763216	31779	12	10873679	c/g	c	S
668521	32194	12	10874094	a/c	c	M
669503	32441	12	10874341	t/c	c	Y
3906864	32454	12	10874354	t/c	g	R
3906863	32459	12	10874359	c/t	a	R
7957888	35151	12	10877051	a/t	t	W
9300230	35362	12	10877262	a/t	t	W
7306214	35630	12	10877530	g/c	g	S
763839	36930	12	10878830	g/c	g	S
2418105	37490	12	10879390	g/a	g	R
666841	38432	12	10880332	c/t	c	Y
3851578	38688	12	10880588	a/g	g	R
7138797	39524	12	10881424	t/c	c	Y
7295252	41580	12	10883480	a/c	a	M
2418106	42531	12	10884431	t/c	t	Y
7299578	42665	12	10884565	a/g	a	R
621112	43038	12	10884938	a/g	a	R
3863320	44183	12	10886083	g/a	g	R
1373432	46271	12	10888171	a/t	t	W
1047699	49075	12	10890975	c/t	t	Y
1063193	49147	12	10891047	c/t	c	Y
2232959	49180	12	10891080	c/g	g	S
2227296	50301	12	10892201	a/g	a	R
1548804	50773	12	10892673	g/a	g	R
2232958	51243	12	10893143	g/a	c	Y
2232957	51530	12	10893430	t/c	a	R
2232956	52107	12	10894007	a/g	c	Y
1972571	52821	12	10894721	a/g	a	R
3759250	53341	12	10895241	t/c	a	R
3759249	53376	12	10895276	c/g	g	S
1541525	54047	12	10895947	c/t	t	Y
2098248	54392	12	10896292	t/c	t	Y
2900550	54482	12	10896382	t/a	a	W
7302130	55468	12	10897368	c/a	a	M
4763583	56990	12	10898890	a/g	a	R
4360778	57712	12	10899612	a/t	a	W
1607695	59667	12	10901567	c/t	a	R
1607694	59684	12	10901584	t/c	g	R
2192139	62043	12	10903943	a/c	a	M
7978300	63293	12	10905193	t/c	t	Y
7397871	63485	12	10905385	g/t	g	K
4763217	63778	12	10905678	c/t	t	Y
2159900	64222	12	10906122	a/g	a	R
10772370	65722	12	10907622	a/g	a	R

dbSNP	Position in SEQ ID NO:2	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
7398682	66315	12	10908215	c/t	c	Y
2900551	66829	12	10908729	a/g	a	R
2900552	66966	12	10908866	c/t	c	Y
2418214	66971	12	10908871	a/c	c	M
2418215	67013	12	10908913	t/c	c	Y
965243	70375	12	10912275	a/t	t	W
1117548	74118	12	10916018	t/a	t	W
1520225	75224	12	10917124	c/t	c	Y
1520226	75236	12	10917136	t/c	t	Y
1520227	75246	12	10917146	g/c	g	S
971919	75812	12	10917712	c/a	a	M
2159901	78968	12	10920868	c/t	t	Y
2159902	78998	12	10920898	t/c	t	Y
2110099	79328	12	10921228	a/c	c	M
7314847	80922	12	10922822	t/c	t	Y
7296003	81055	12	10922955	t/c	c	Y
4281556	81412	12	10923312	g/a	a	R
4763219	81785	12	10923685	a/g	g	R
3851579	82079	12	10923979	g/a	a	R
3851580	82087	12	10923987	g/a	g	R
1049119	82958	12	10924858	a/g	c	Y
2298866	83351	12	10925251	t/g	a	M
2298865	83442	12	10925342	c/t	a	R
2298864	83472	12	10925372	a/c	t	K
2298863	83966	12	10925866	a/g	t	Y
3180393	84414	12	10926314	t/c	t	Y
2070837	86563	12	10928463	c/g	c	S
7956204	86788	12	10928688	g/a	g	R
2418216	86796	12	10928696	t/g	t	K
3741844	87634	12	10929534	g/a	c	Y
4262798	88530	12	10930430	g/a	a	R
2418217	89202	12	10931102	a/g	a	R
2418218	89632	12	10931532	t/c	t	Y
7137492	89697	12	10931597	t/c	t	Y
2110100	89723	12	10931623	g/a	g	R
1013312	91063	12	10932963	c/g	c	S
4579993	91335	12	10933235	c/g	g	S
1013313	91504	12	10933404	t/c	t	Y
7397106	91619	12	10933519	c/t	t	Y
2215716	93715	12	10935615	a/g	g	R
2192140	93945	12	10935845	t/c	c	Y
4763589	94235	12	10936135	t/c	t	Y
1468697	95851	12	10937751	t/c	c	Y
2070837	130860	12	10972760	c/g	c	S
3180393	132815	12	10974715	t/c	a	R
2298865	133778	12	10975678	c/t	t	Y

Assay for Verifying and Allelotyping SNPs

[0235] The methods used to verify and allelotype the proximal SNPs of Table 12 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 13 and Table 14, respectively.

TABLE 13

dbSNP rs#	Forward PCR primer	Reverse PCR primer
523051	CTCTGCTCAGAGCATAGATG	TTCCATGCATTTTAACCCCG
693620	GTAGGTCAATAAAAGAGGAGG	TTAGGCCGAGATTTTCAGAG
2588349	GTTCAAGAGTTTGATGTCAAG	CAGGGCATCTAAATTGAACG
2588350	TGTATCTGGGACATACCATC	GTTAGTACAGGGTTGATCAC
619381	AAAGCATAGTTTCTCTTCAG	GCATCTCTAAAGGTGATTTGG
3759252	GCTGTGATTTTTGGTGAGTC	TATTGTTCCCCAGTATTAGG
3759251	TTGTCCTTTCTCATTGCCAC	ATTAGAGCTATGGACTCACC
2418107	AGGTAAATGGGCATACAGTG	TGAAGACTGGAGCTATCTTC
7303054	AGGGAATTGGCCCAATTTGC	CCATTTACCTACTCAGCAGG
1838345	CCCAGGTTCAAGTGATTCTC	AATTAGCCAGGCATGGTTGC
620878	CCAGACCAAACGTTTCAATAA	AAGAAGAAACGAATGTTTAC
2537817	GTCAGAATGCTGACATGTAG	GGGGAAAAATGAGAATAGAC
1548803	ATGGGACAATTGCAAACAGG	ACTGAAATGTTCCATGTGAG
667123	TAAATACAAGAAGCCCTAGG	AGGGAAAGTACAACTTAGCC
1838346	GTTAGAAGGCTAGAGAGAAC	TTCATGAGTACTCTAGCTGG
2159903	AGTCAGGATACTCTTTAGGG	GGGAAGCTTTTCTGAAGATG
3944035	GGGTCTCTATGGAACAAAAG	TGAGGGAAGCTTTTCTGAAG
3741845	TGCTTGGTGTGTCTAACTAG	CAAACAGTTAACCCTGAACC
2110096	ATCAAGAACAGGATACTGCG	GGGTAGGAGATAAAGTCAGC
759055	CCCCTATCTTTTTGTTGGATG	GCTCTGTTTTCATGAGGTTC
589377	TTCACTGTTCAGATTGCTGG	ACTCCAGTTGTAGGTAGAAG
7960194	TGCTATCTTCCATGGAAGAA	TTTTTTTCCCTGTATGCCTC
7978242	GACGCTAAGCATCATTAGTC	TAGCCATTCTAGTGGATGTG
601051	TTTTTACGTCTGTGTGAACC	CACAGAAACCGAAAACCAAC
4262797	ATTTTAGTGCACCCATCACC	GTCTTTAGAGACTCAAAAGGG
2215714	GAGTTAAACATCAGTCGTTGT	TTATTATATAACGTAGGAA
1373434	CGTTTAAGATGATAGATCTTC	CTACGCAATAGGCTATTTTC
2215715	ACATGGATGGAAAGGAAGAC	TATGAGCGAGAATATGTGGC
612456	TATCAACAAGCCAAGATCCC	CTTTAGCCTTTTGTTGGACG
612808	GGCTGAGTGTTAAGTATTCC	AAGGCAAACCCTTAATAGGC
689118	GAAATGTTCTGTCTTCAGGC	AACGTTTCTGTGAGTGCAAG
597468	TACATGTTGAGATCCCAGAC	TGCATACATTGCGAAGATTC
592864	AAATTGTGTTTCTTGTAAGC	AATGAACTAAATACTCCAAG
640372	ATCTGAAACTCTTTGAGCGC	TCCAAAATGTTCCAATGAGC
7986559	CCTAATTCTTTTCTATCGGTG	GAAGCTAGAAAGCTAAGAGTG
654834	CTGTCCTTTTTCTGGCTTTC	CAAACAAAATAGAGTCCAGCG
4763216	TTGTAACCATAGAAAGCAGG	TCCCTCTATTTTTCTACTAC
668521	TGCTGCTGATGTAACTGCAG	GTGCAATAGTAACTGGGCTC
669503	GTAATCCACTCATTCAGATC	TACCTAGAGGAAATAAGTGG
3906864	TTTCTAACATCCACCTTCTG	GGTGTGAATAAGTTGTAATCC
3906863	CAGGACTGACCTCTAAAATAG	ACATCCACTTTCTGTAGATC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
7957888	ACCTTAACAAAGTTCTGTGG	GAGCCACGAAAAAAAAAATG
9300230	CCAGACAAGCTAAAATACAG	CAAATACACACAGACACATC
7306214	CTATAGCACAGTAGGGTGAC	TCTTCGTGTTGGGCACATTC
763839	TATTCATGTAACACCGAAGG	GGAACAGCAAACTGAAACAG
2418105	GGAGGCATTCCATTCTTTTC	GTGTATCCAAAGCTTTAGTG
666841	TAATCCCCAAGAGAGAGGAC	AACTAACACAGATGGCCACC
3851578	GTGTGTATTTGCCCTTCTGC	GGCATGTGATAGGAATGTGC
7138797	GTTCTGAAGTACATGTGCAG	TTAATACCTAGGTGATGGGC
7295252	CAAGTTTACAATCACAGCTG	GGTAAATGTATTTGACAGTTG
2418106	CAGCCCAGAATGGCTTTGAA	ACTAACGATGGGTGATGAGC
7299578	ATCTGCCCCATGATCCAATC	CCCCTGAAAACCTCATGTTG
621112	TATGTTGGGTCACAAACCTG	AGCAAGGCGTACAAAACAAC
3863320	GTTGCTCACAATACTGGAGG	TGTCTTCAGCAGAACTCATC
1373432	TAAGGCAGAGGGCTACTTAC	GCATTTTACAAGACTTAAATCG
1047699	ATTACCAGAGTGGTTGCTCC	ATTTCCTTCTGTCAGCCTGC
1063193	AGGCTGACAGAAGGAAATCG	TCAGCAGAGACCACCAAAAC
2232959	TCAGCAGAGACCACCAAAAC	GGTGGTCGTTGCTGATTTTG
2227296	TGAGAAGAAGACACTGAAGG	ATCCCAATGATCCTCAATCC
1548804	CCCATTTTTCTTCCAATCAC	GGAGCAACCAGGAGTAAATA
2232958	TCTTCTCAACCTCCTTCCTC	ATGACTCTTCTAGGGCCTCA
2232957	TTTATAAACATGAGCAGAAC	CGCATTCAACTTTATGAGAGG
2232956	GCCAATTTTGCCTTACTAACC	CTAGCATTAACCAGAGATAC
1972571	ATCACCAGCTGCCACTTTTC	CCTCAAATTATCACCAGCCC
3759250	TTCTGCCTAGGTGGCTTATG	AATCATGTCTGCATGGCACG
3759249	CACACAGTGAATCATGTCTG	ATGGTAGACCATCAGGTAGG
1541525	TATCTGTAGACGTGCATCCC	CTGACACCTGTCCTCATAAG
2098248	GGGAGAATCATGGCTTGA	GTACTGCCCTTAGCAAATCC
2900550	TCAACAGTCTCCAGCTGATC	ACTCTGGCTGTTGTAAGGAG
7302130	TACACCTGTGAGCACTGACA	CCTTTTCTCAAGGTGTGTGC
4763583	AATGGTTTAGCACCATCCCC	GGGAGGTGACACACACTTTT
4380778	AGGCTGCAAGTTTTCCAAAC	GCCTAAGCAAAGAAATGAGC
1607695	TTCTTCTCACTGACCTATTC	ATCAAACAGAAACGCTGGAG
1607694	ATCAAACAGAAACGCTGGAG	CTCTTTTTTTGTCTGACTGGG
2192139	GGTCTTTTGTTTGCTAGGAC	GTAACCTGCCAAGATTGAAC
7978300	TCTGAGTCTTTTGTGGTACC	TGCAGATTCAATGCAATCCC
7397871	AGGAGCTGAAACGATGGTTC	CAAAATGGCATGACACTGGC
4763217	ACATTGTGTCTGTGCTTCTC	GCAGTCTCTTATTGTCTGAG
2159900	TTACAACAAGTGCTCAAGGG	GTGTCTTCTCTAACAGTGAG
10772370	GAATGACCATATGATCCAGC	TAGGTATCCCTTTGATGTCC
7398682	GACAGATACCCTAACTTGATC	GCAGTAAAGCTATCAATCCTG
2900551	GTACCTCAACACAAAAAGGC	GAGGAAATCTTTCACCTTTCC
2900552	GCTAGATATACTAGGCAAGAG	TGTCATCCGAAAAGATGAAC
2418214	TGTCATCCGAAAAGATGAAC	GCTAGATATACTAGGCAAGAG
2418215	CTATTCTGCAACTTTACCAG	TAGAAGGAAGTCAATTGTTC
985243	GGCTGTGTAGGTTTGTCCTC	TTCATCCCATTCCATCCAGC
1117548	GTGGTACATCACATTAACAG	TTCCTTCTTTGTGTAGTTCG
1520225	CGTGACTCTCTGTACAGCAT	CAGCACTAGGCTGTGAAAAG
1520226	TAGGGTAAAATGTGCACAGC	GCATTGTTAGTGGTTGCTCC
1520227	TAGGGTAAAATGTGCACAGC	GCATTGTTAGTGGTTGCTCC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
971919	TTAGTGACCTTCATAGAACC	CAGGCATTCTTAGAAGACAG
2159901	CATATCTACTTGTGAACTGC	GAAGGTAAAACCACTGATGC
2159902	CCCCTATAAGAGCAAAATAC	GAACTTTTCAGAAAGCATCAG
2110099	ATTCCTTCTCAACCCACATC	ACATTACAGGAAGGCCTTTC
7314847	CCTCTGCATAGATACAGTAG	CTCTTATACACCCTGATTGG
7296003	TGGTTACTTAGGTTGATGCC	CTACTGTATCTATGCAGAGG
4281556	GGCCTTAGGGTTTCCATAAC	AAGCCTTGGAAGAGTGAGTG
4763219	AGGAGAATCGCTTGAACCTG	TGGAGTCTCATTCTGTCACC
3851579	AGGCTTTCAAACAAAATAGG	TTACGGGTCTGATAAGAAAG
3851580	AGGCTTTCAAACAAAATAGG	TTACGGGTCTGATAAGAAAG
1049119	GATAACAGTGTTTCAAATGC	GAAATTGCAAGCTGATTGTT
2298866	TAACCAATCCCTGTCACTGG	ACAAAGATGGGCACTGCAAC
2298865	CCTGAGTTCATTTAGATCTC	ACGGAACAACCAAACAATAG
2298864	CCAGTAAACACTGAAGAGATC	GATTTGTGTCTTACTCACTGG
2298863	AGCTCTTGAAGGCAATTCTG	TATGTCCTCATTGTCAACCC
3180393	AACAAGGTCCACCACCTCCT	TTGTGGCCTTCCTTGAGGAG
2070837	ACACACCCACACAAACTCAC	TGGAAAGACTGCTATTCTGC
7956204	TTTGGACAATTCTTGTAGCC	TGTGAGATGTGTGAGGACAG
2418216	CCCAGGCACACATATATGAG	GCCTGAGTGTAGTGAGATTC
3741844	GCTGATTGCTCTGTGATACC	AAGTGCAGCTGGTGATTCTG
4262798	TCTAGGTACCCAAGCTCCTG	AAGAGCTGAAAGGGACACTG
2418217	GCCTGGAACACTAAAGATGG	CTCACTGACTCTCAGAGAAG
2418218	AAATTAGTCAGTCATGGTGG	TTCAAGCAATTCTCCTGCCT
7137492	TCCTCATTTACTACAGTGAC	CCCATCTCTACTAAAAATAC
2110100	CTGGAATTACAAAGAGAAGAG	CCCATCTCTACTAAAAATAC
1013312	ATTGTGTTTGCCCACTTTCC	GTGGAACATCAAGAATGAAG
4579993	ATTTGTGCTCCTTTCTACTG	AGCAGAAAGAAGGAAATGAC
1013313	TTCCAAACACAGCAAAGAGC	TTGGTTGTATTCTGGTTGGG
7397106	TCTGAAACTCAGAATGCATG	ATACTCTAAACAATACAGGG
2215716	GTACTAGCAGTAGTCAGAGG	GACACCACTACTTGCACATG
2192140	CCTTGGGATTTAGAAATAGGG	GTAAGCAAATATCTCTGGAG
4763589	TTGGCAACTGTGTAACCTTG	TCAAACATACTGTTTGCTCAC
1468697	GAACACAATCGCAAGTTTAAG	GTTCTTCAAAATCTGCTTCC
2070837	ACACACCCACACAAACTCAC	TGGAAAGACTGCTATTCTGC
3180393	AACAAGGTCCACCACCTCCT	TTGTGGCCTTCCTTGAGGAG
2298865	CCTGAGTTCATTTAGATCTC	ACGGAACAACCAAACAATAG

TABLE 14

dbSNP rs#	Extend Primer	Term Mix
523051	TCAGAGCATAGATGATGGCAA	ACT
693620	AGAGGAGGAAACCTTAATTTCT	ACT
2588349	AGAGTTTGATGTCAAGGAAATG	ACG
2588350	TACCATCAAAAGCACATCATTC	ACT
619381	GAATTTTCTTCCTTTTAGAATAGA	ACG

dbSNP rs#	Extend Primer	Term Mix
3759252	TGGTGAGTCCATAGCTCTAAT	CGT
3759251	CCAGCTACTTTATGCCAGAG	CGT
2418107	TGGCCAGGCCAAGGATCG	ACT
7303054	CAGCCTAAATTAACCGTATGTG	ACT
1838345	CCTGCCTGAGCCTGCCAA	ACT
620878	TTCAATAATATTATAGTGAGGATG	ACT
2537817	GACATGTAGAAAAATTGCCTGC	ACT
1548803	TTGCAAACAGGTTAAAGAGAGT	ACG
667123	AAGCCCTAGGGTATTGTGATT	ACG
1838346	AAGGCTAGAGAGAACATTCCA	ACT
2159903	AGGGGTCTCTATGGAACAAAA	ACT
3944035	AACAAAAGGCTTTCTTCTTCTAA	ACT
3741845	CAAAGGATAAAGGGAACCATC	ACT
2110096	CAGGATACTGCGATACTGTC	ACG
759055	GTAATCTTGTTTGTTGTATTTTTC	CGT
589377	CTGGTGTGTAGAAACACAACA	ACT
7960194	GCCCAGGGCTCAGGGAAG	CGT
7978242	GCAAACCAAAACCACAGTCAC	ACT
601051	CCCGCTTATAAGCAAGAGCA	ACG
4262797	TTATCCCTATCCTCCCTTCC	ACT
2215714	GCATCACTCTTTCTACGCAATA	ACT
1373434	TGTTTCAAAACATCACTATGTAC	ACG
2215715	GGAAAGGAAGACATTATGGTAC	ACG
612456	CCAAGATCCCTGAAAGGCAAA	CGT
612808	CTACTTTAGCCTTTTGTTGGAC	ACT
689118	GCTTCATACACACACACACAT	ACT
597468	CCCAGACTGTGTCATTCTTC	ACT
592864	GTGTTTCTTGTAAGCAGTATAC	ACT
640372	AAACTCTTTGAGCGCTGACAT	ACT
7966559	TCTATCGGTGATTTCTGCTGT	CGT
654834	CACAATTGGCTTTTAAAACTCC	ACT
4763216	CCATAGAAAGCAGGACTGGTT	ACT
668521	AAATTAGTCTAGAACTTGTGGAA	ACT
669503	CCACTCATTCAGATCTACAGAA	ACT
3906864	ACATCCACCTTCTGTAGATCT	ACT
3906863	GTGTGAATAAGTTGTAATCCAC	ACG
7957888	TGTGGAAATATTTGAAGACTCTT	CGT
9300230	ATACAGAGAAGTAGAGGACAAA	CGT
7306214	CACAGTAGGGTGACTACAATTA	ACT
763839	TAACACCGAAGGTTCCTCAG	ACT
2418105	CATTCTTTTCCAGTGTCAATCAA	ACG
666841	GGACTTGGCAGCATTATTTATTA	ACG
3851578	CAGCTAATTGTGCTCCCTCA	ACT
7138797	AAGTACATGTGCAGAATGTGC	ACT
7295252	ATCACAGCTGATAATGTCATAAT	ACT
2418106	GAATGGCTTTGAATATGACCCA	ACT
7299578	CAGGTGCTATCACAACATCG	ACT
621112	CACAAACCTGCCATTTTACTTT	ACT

dbSNP rs#	Extend Primer	Term Mix
3863320	AATACTGGAGGCTGGAAGAC	ACG
1373432	CAGAGGGCTACTTACAAGAATT	CGT
1047699	TCTTGCTGGTCTGTCCCTC	ACG
1063193	AGTTGACGGTGTCCTCGT	ACG
2232959	ATCACCGCCATCCTCCCC	ACT
2227296	GAAATTCTAGTGGAAAAAG	ACT
1548804	AAAATTTCAATATGTTGCAGGCAG	ACG
2232958	CCTCCCCTCCTGCTCTTC	ACG
2232957	GGACCTCACCACCTGAAAG	ACT
2232956	TGCCTTACTAACCTCCAGG	ACT
1972571	CTGCCACTTTTCATTACAGGC	ACT
3759250	TAGGTGGCTTATGGTAGACC	ACT
3759249	TCTGCATGGCACGCCCCT	ACT
1541525	CCCTCTGTGGTCTGTGCTAA	ACG
2098248	GAATCATGGCTTGACTCAGG	ACT
2900550	GCCCCTCACCACATCCCA	CGT
7302130	CCCACCCAGCAGGAAGAC	CGT
4763583	CCTGCGTACTATTCTTATGAC	ACT
4360778	TTTTACACTCTGCTTCCCTTTT	CGT
1607695	TTCTCACTGACCTATTCTATTTT	ACG
1607694	AGCTTCAAAAATAGAATAGGTCA	ACT
2192139	CTTTGATCTCATCACTTGTTATT	ACT
7978300	TTCTGTTTCTGTGAAGAATGTC	ACT
7397871	GCCAGGCTCACAGTCCAAA	CGT
4763217	TTGGACGTAGGACAAAGAACT	ACG
2159900	GCTCAAGGGAGTTCTACATCT	ACT
10772370	GACCATATGATCCAGCAATTTC	ACT
7398682	GCTTATAACAAAATATCATGTGTC	ACG
2900551	GCCATATATGACAAACCCACA	ACT
2900552	ATATACTAGGCAAGAGATAGAAA	ACG
2418214	CTTCCTTCTATTCAATTTCAATTT	ACT
2418215	CTATAAATAAGATCATGTCATCC	ACT
965243	CAGACCCATCAGAGAAGTAC	CGT
1117548	CCAAATAAGCATATGAATAGATG	CGT
1520225	CAGCATTGTTAGTGGTTGCTC	ACG
1520226	GCTGTGAAAAGCTATGTATGC	ACT
1520227	CAGCACTAGGCTGTGAAAAG	ACT
971919	ATAAGATAGGTCTGTTGGAAAG	CGT
2159901	TGATTTCTTCAGCCATTATCCA	ACG
2159902	TTAAGCTTGCATGAAAGAAGGT	ACT
2110099	TTATGTCAGCAGAAAAACAGAAA	ACT
7314847	GAGTTTTAGTTCTTTGAGAAATC	ACT
7296003	GGTTGATGCCATATCTTTGCT	ACT
4281556	GGTTTCCATAACAAGAATAACAAA	ACG
4763219	CCGATATCTTGCCACTGCAG	ACT
3851579	CAAAATAGGAACACACTGAGTAT	ACG
3851580	CTTTCAAACAAAATAGGAACACA	ACG
1049119	TGGCATCATGCTCTAACTTCA	ACT

dbSNP rs#	Extend Primer	Term Mix
2298866	CCTGTCACTGGATATTAAGGC	ACT
2298865	CATTTAGATCTCTTCAGTGTTTA	ACG
2298864	CTGAAGAGATCTAAATGAACTCA	ACT
2298863	CAATTCTGATTTTGAGAATCACT	ACT
3180393	GCCACAAGGACCACCCCA	ACT
2070837	CCACACAAACTCACATACACA	ACT
7956204	AGGCTGCCAGCACCTTTCT	ACG
2418216	GCACACATATATGAGAGAAAGG	ACT
3741844	GTGATACCTAGAAATTCCCTG	ACG
4262798	CGTCTCCCTTTCACCCACC	ACG
2418217	TGCCAGGAAAGATGAACATTG	ACT
2418218	CAGTCATGGTGGCGAGGG	ACT
7137492	GCCATGTGACTTAATTTCTTTAG	ACT
2110100	TCCTCATTTACTACAGTGACC	ACG
1013312	GCCCACTTTCCCCCTTTCT	ACT
4579993	ACTGTTATTTCTGGTTTCTGGT	ACT
1013313	ACAGCAAAGAGCATAAACCTAC	ACT
7397106	CTCAGAATGCATGAATAGTACA	ACG
2215716	CGTACTATTTCTTCAAGCTTCA	ACT
2192140	TAGGGTATCCCTCTTGGTCA	ACT
4763589	CTGTGTAACCTTGATCTTGGG	ACT
1468697	CAATCGCAAGTTTAAGGTAACA	ACT
2070837	CCACACAAACTCACATACACA	ACT
3180393	GCCACAAGGACCACCCCA	ACT
2298865	CATTTAGATCTCTTCAGTGTTTA	ACG

Genetic Analysis

[0236] Allelotyping results are shown for female cases and controls in Table 15. The allele frequency for the A2 allele is noted in the fifth and sixth columns for control pools and case pools, respectively, where "AF" is allele frequency. Some SNPs do not have an allele frequency disclosed because of failed assays.

TABLE 15: Female Allelotyping Results

dbSNP rs#	Position in SEQ ID NO:2	Chromo-some Position	A1/A2 Allele	Controls (high BMD) AF	Cases (Low BMD) AF	p-Value	OR	Low BMD Associate d Allele
523051	229	10842129	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
693620	368	10842268	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
2588349	583	10842483	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
2588350	2424	10844324	T/C	T=0.34	T=0.28	0.0295	0.76	C
				C=0.66	C=0.72			
619381	3625	10845525	C/T	C=0.86	C=0.91	0.0065	1.67	C

dbSNP rs#	Position in SEQ ID NO:2	Chromo-some Position	A1/A2 Allele	Controls (high BMD) AF	Cases (Low BMD) AF	p-Value	OR	Low BMD Associated Allele
				T=0.14	T=0.09			
3759252	3709	10845609	C/A	C=1.00	C=1.00			
				A=0.00	A=0.00			
3759251	3750	10845650	T/A	T=0.01	T=0.01			
				A=0.99	A=0.99			
2418107	4724	10846624	G/C	G=1.00	G=1.00			
				C=0.00	C=0.00			
7303054	4781	10846681	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
1838345	6161	10848061	A/G	A=	A=			
				G=	G=			
620878	7097	10848997	T/G	T=0.07	T=0.03	0.0098	0.37	G
				G=0.93	G=0.97			
2537817	8025	10849925	T/C	T=0.19	T=0.16	0.4485	0.85	C
				C=0.81	C=0.84			
1548803	8398	10850298	C/T	C=0.47	C=0.41	0.0564	0.78	T
				T=0.53	T=0.59			
667123	10144	10852044	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
1838346	10384	10852284	A/G	A=0.76	A=0.80	0.1329	1.26	A
				G=0.24	G=0.20			
2159903	11116	10853016	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
3944035	11132	10853032	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
3741845	11482	10853382	T/C	T=0.54	T=0.49	0.0877	0.81	C
				C=0.46	C=0.51			
2110096	14544	10856444	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
759055	15688	10857588	T/A	T=0.45	T=0.37	0.0084	0.72	A
				A=0.55	A=0.63			
589377	17311	10859211	G/C	G=0.01	G=0.00			
				C=0.99	C=1.00			
7960194	17831	10859731	G/T	G=1.00	G=1.00			
				T=0.00	T=0.00			
7978242	20012	10861912	A/G	A=0.62	A=0.68	0.0611	1.27	A
				G=0.38	G=0.32			
601051	21997	10863897	G/A	G=0.01	G=0.00			
				A=0.99	A=1.00			
4262797	22861	10864761	A/G	A=0.61	A=0.54	0.0391	0.76	G
				G=0.39	G=0.46			
2215714	23470	10865370	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
1373434	23515	10865415	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
2215715	23863	10865763	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
612456	24108	10866008	C/A	C=0.03	C=0.02			

dbSNP rs#	Position In SEQ ID NO:2	Chromo-some Position	A1/A2 Allele	Controls (high BMD) AF	Cases (Low BMD) AF	p-Value	OR	Low BMD Associated Allele
				A=0.97	A=0.98			
612808	24138	10866038	T/C	T=0.20	T=0.11	0.0001	0.52	C
				C=0.80	C=0.89			
689118	26469	10868369	T/C	T=0.68	T=0.71	0.3485	1.15	T
				C=0.32	C=0.29			
597468	27769	10869669	A/G	A=0.99	A=1.00			
				G=0.01	G=0.00			
592864	29683	10871583	T/C	T=0.10	T=0.11	0.9292	1.04	T
				C=0.90	C=0.89			
640372	30491	10872391	A/C	A=0.96	A=0.96	0.6780	1.21	A
				C=0.04	C=0.04			
7966559	30745	10872645	A/T	A=0.79	A=			
				T=0.21	T=			
654834	31429	10873329	T/C	T=0.04	T=0.03	0.3922	0.74	C
				C=0.96	C=0.97			
4763216	31779	10873679	C/G	C=0.50	C=0.45	0.0951	0.82	G
				G=0.50	G=0.55			
668521	32194	10874094	A/C	A=0.98	A=0.99			
				C=0.02	C=0.01			
669503	32441	10874341	T/C	T=0.96	T=0.96	0.4685	1.26	T
				C=0.04	C=0.04			
3906864	32454	10874354	T/C	T=0.99	T=1.00			
				C=0.01	C=0.00			
3906863	32459	10874359	C/T	C=0.34	C=0.41	0.0288	1.35	C
				T=0.66	T=0.59			
7957888	35151	10877051	A/T	A=0.87	A=0.93	0.0155	2.12	A
				T=0.13	T=0.07			
9300230	35362	10877262	A/T	A=1.00	A=1.00			
				T=0.00	T=0.00			
7306214	35630	10877530	G/C	G=1.00	G=1.00			
				C=0.00	C=0.00			
763839	36930	10878830	G/C	G=0.82	G=0.88	0.0047	1.61	G
				C=0.18	C=0.12			
2418105	37490	10879390	G/A	G=0.21	G=0.16	0.0198	0.69	A
				A=0.79	A=0.84			
666841	38432	10880332	C/T	C=0.89	C=0.93	0.0180	1.72	C
				T=0.11	T=0.07			
3851578	38688	10880588	A/G	A=0.89	A=0.93	0.0243	1.67	A
				G=0.11	G=0.07			
7138797	39524	10881424	T/C	T=0.97	T=0.97	0.9815	0.99	T
				C=0.03	C=0.03			
7295252	41580	10883480	A/C	A=1.00	A=1.00			
				C=0.00	C=0.00			
2418106	42531	10884431	T/C	T=0.35	T=0.30	0.1784	0.80	C
				C=0.65	C=0.70			
7299578	42665	10884565	A/G	A=0.30	A=0.23	0.0036	0.67	G
				G=0.70	G=0.77			
621112	43038	10884938	A/G	A=0.87	A=0.92	0.0086	1.69	A
				G=0.13	G=0.08			

dbSNP rs#	Position in SEQ ID NO:2	Chromo-some Position	A1/A2 Allele	Controls (high BMD) AF	Cases (Low BMD) AF	p-Value	OR	Low BMD Associated Allele
3863320	44183	10886083	G/A	G=0.52	G=0.49	0.2271	0.87	A
				A=0.48	A=0.51			
1373432	46271	10888171	A/T	A=0.00	A=0.00			
				T=1.00	T=1.00			
1047699	49075	10890975	C/T	C=0.79	C=0.85	0.0062	1.53	C
				T=0.21	T=0.15			
1063193	49147	10891047	C/T	C=0.75	C=			
				T=0.25	T=			
2232959	49180	10891080	C/G	C=1.00	C=1.00			
				G=0.00	G=0.00			
2227296	50301	10892201	A/G	A=0.77	A=0.77	0.9934	1.00	A
				G=0.23	G=0.23			
1548804	50773	10892673	G/A	G=0.24	G=0.15	0.0005	0.54	A
				A=0.76	A=0.85			
2232958	51243	10893143	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
2232957	51530	10893430	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
2232956	52107	10894007	A/G	A=0.87	A=0.92	0.0170	1.64	A
				G=0.13	G=0.08			
1972571	52821	10894721	A/G	A=0.13	A=0.13	0.8117	1.04	A
				G=0.87	G=0.87			
3759250	53341	10895241	T/C	T=0.99	T=1.00			
				C=0.01	C=0.00			
3759249	53376	10895276	C/G	C=1.00	C=1.00			
				G=0.00	G=0.00			
1541525	54047	10895947	C/T	C=0.00	C=0.00			
				T=1.00	T=1.00			
2098248	54392	10896292	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
2900550	54482	10896382	T/A	T=0.89	T=0.93	0.0535	1.59	T
				A=0.11	A=0.07			
7302130	55468	10897368	C/A	C=0.36	C=0.36	0.9550	1.01	C
				A=0.64	A=0.64			
4763583	56990	10898890	A/G	A=	A=			
				G=	G=			
4360778	57712	10899612	A/T	A=0.00	A=0.01			
				T=1.00	T=0.99			
1607695	59667	10901567	C/T	C=0.00	C=0.01			
				T=1.00	T=0.99			
1607694	59684	10901584	T/C	T=0.81	T=0.83	0.3469	1.17	T
				C=0.19	C=0.17			
2192139	62043	10903943	A/C	A=0.30	A=0.28	0.4555	0.89	C
				C=0.70	C=0.72			
7978300	63293	10905193	T/C	T=0.36	T=			
				C=0.64	C=			
7397871	63485	10905385	G/T	G=0.00	G=0.00			
				T=1.00	T=1.00			
4763217	63778	10905678	C/T	C=0.89	C=0.90	0.8698	1.05	C

dbSNP rs#	Position in SEQ ID NO:2	Chromo-some Position	A1/A2 Allele	Controls (high BMD) AF	Cases (Low BMD) AF	p-Value	OR	Low BMD Associated Allele
				T=0.11	T=0.10			
2159900	64222	10906122	A/G	A=0.02	A=0.02			
				G=0.98	G=0.98			
10772370	65722	10907622	A/G	A=0.31	A=0.27	0.1478	0.82	G
				G=0.69	G=0.73			
7398682	66315	10908215	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
2900551	66829	10908729	A/G	A=0.00	A=0.01			
				G=1.00	G=0.99			
2900552	66966	10908866	C/T	C=0.61	C=0.59	0.5150	0.92	T
				T=0.39	T=0.41			
2418214	66971	10908871	A/C	A=0.51	A=0.57	0.0565	1.27	A
				C=0.49	C=0.43			
2418215	67013	10908913	T/C	T=0.82	T=0.86	0.1232	1.29	T
				C=0.18	C=0.14			
965243	70375	10912275	A/T	A=0.82	A=0.85	0.1637	1.25	A
				T=0.18	T=0.15			
1117548	74118	10916018	T/A	T=1.00	T=0.99			
				A=0.00	A=0.01			
1520225	75224	10917124	C/T	C=0.27	C=0.22	0.0956	0.79	T
				T=0.73	T=0.78			
1520226	75236	10917136	T/C	T=0.99	T=0.98			
				C=0.01	C=0.02			
1520227	75246	10917146	G/C	G=0.91	G=0.82	0.00001	0.46	C
				C=0.09	C=0.18			
971919	75812	10917712	C/A	C=0.00	C=0.00			
				A=1.00	A=1.00			
2159901	78968	10920868	C/T	C=0.99	C=0.99			
				T=0.01	T=0.01			
2159902	78998	10920898	T/C	T=0.54	T=0.51	0.3599	0.90	C
				C=0.46	C=0.49			
2110099	79328	10921228	A/C	A=0.60	A=0.63	0.4080	1.11	A
				C=0.40	C=0.37			
7314847	80922	10922822	T/C	T=0.51	T=0.49	0.6059	0.94	C
				C=0.49	C=0.51			
7296003	81055	10922955	T/C	T=0.63	T=0.65	0.4799	1.09	T
				C=0.37	C=0.35			
4281556	81412	10923312	G/A	G=0.85	G=0.87	0.5327	1.17	G
				A=0.15	A=0.13			
4763219	81785	10923685	A/G	A=	A=			
				G=	G=			
3851579	82079	10923979	G/A	G=	G=0.34			
				A=	A=0.66			
3851580	82087	10923987	G/A	G=0.56	G=0.54	0.5618	0.93	A
				A=0.44	A=0.46			
1049119	82958	10924858	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
2298866	83351	10925251	T/G	T=0.72	T=0.69	0.3240	0.87	G
				G=0.28	G=0.31			

dbSNP rs#	Position in SEQ ID NO:2	Chromo-some Position	A1/A2 Allele	Controls (high BMD) AF	Cases (Low BMD) AF	p-Value	OR	Low BMD Associated Allele
2298865	83442	10925342	C/T	C=0.95	C=0.97	0.1804	1.67	C
				T=0.05	T=0.03			
2298864	83472	10925372	A/C	A=0.36	A=0.34	0.5936	0.93	C
				C=0.64	C=0.66			
2298863	83966	10925866	A/G	A=1.00	A=1.00			
				G=0.00	G=0.00			
3180393	84414	10926314	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
2070837	86563	10928463	C/G	C=0.43	C=0.43	0.9403	0.99	C
				G=0.57	G=0.57			
7956204	86788	10928688	G/A	G=0.86	G=			
				A=0.14	A=			
2418216	86796	10928696	T/G	T=1.00	T=1.00			
				G=0.00	G=0.00			
3741844	87634	10929534	G/A	G=0.50	G=0.48	0.3827	0.90	A
				A=0.50	A=0.52			
4262798	88530	10930430	G/A	G=0.60	G=0.62	0.4130	1.10	G
				A=0.40	A=0.38			
2418217	89202	10931102	A/G	A=0.53	A=0.52	0.6562	0.95	G
				G=0.47	G=0.48			
2418218	89632	10931532	T/C	T=	T=			
				C=	C=			
7137492	89697	10931597	T/C	T=0.75	T=0.76	0.7043	1.07	T
				C=0.25	C=0.24			
2110100	89723	10931623	G/A	G=0.53	G=0.52	0.5403	0.93	A
				A=0.47	A=0.48			
1013312	91063	10932963	C/G	C=0.29	C=0.26	0.1928	0.84	G
				G=0.71	G=0.74			
4579993	91335	10933235	C/G	C=0.00	C=0.00			
				G=1.00	G=1.00			
1013313	91504	10933404	T/C	T=0.61	T=0.58	0.3893	0.90	C
				C=0.39	C=0.42			
7397106	91619	10933519	C/T	C=0.02	C=0.04			
				T=0.98	T=0.96			
2215716	93715	10935615	A/G	A=0.45	A=0.36	0.0372	0.69	G
				G=0.55	G=0.64			
2192140	93945	10935845	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
4763589	94235	10936135	T/C	T=0.53	T=0.52	0.6806	0.95	C
				C=0.47	C=0.48			
1468897	95851	10937751	T/C	T=0.02	T=0.01			
				C=0.98	C=0.99			
2070837	130860	10972760	C/G	C=0.43	C=0.43	0.9403	0.99	C
				G=0.57	G=0.57			
3180393	132815	10974715	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
2298865	133778	10975678	C/T	C=0.95	C=0.97	0.1804	1.67	C
				T=0.05	T=0.03			

[0237] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 2. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 2 can be determined by consulting Table 15. For example, the left-most X on the left graph is at position 10842129. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0238] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0239] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is place at the 3' end of each gene to show the direction of transcription.

Example 5

GRID2 Proximal SNPs

[0240] It has been discovered that a polymorphic variation (rs1948017) in a gene encoding *GRID2* is associated with the occurrence of low BMD (see Examples 1 and 2). One hundred five additional allelic variants proximal to rs1948017 were identified and subsequently allelotyped in low BMD case and high BMD control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 16. The chromosome position provided in column four of Table 16 is based on Genome "Build 34" of NCBI's GenBank.

TABLE 16

dbSNP	Position in SEQ ID NO:3	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
1433661	206	4	94744306	t/c	g	R
1485009	243	4	94744343	a/g	g	R
7681947	2467	4	94746567	t/c	t	Y
1816432	3550	4	94747650	a/g	t	Y
1485018	4994	4	94749094	c/g	g	S
1485017	5167	4	94749267	g/t	c	M
7438397	5193	4	94749293	c/t	c	Y
6834311	5273	4	94749373	g/a	g	R
1368717	5733	4	94749833	a/g	c	Y
1017391	7817	4	94751917	a/c	g	K
2870701	7818	4	94751918	t/a	a	W
7679839	8612	4	94752712	t/g	t	K
1385404	9158	4	94753258	a/g	a	R
1368716	9285	4	94753385	g/a	c	Y
4693316	10680	4	94754780	g/a	a	R
1905707	11866	4	94755966	t/c	c	Y
1905708	11958	4	94756058	a/g	a	R
1905709	12044	4	94756144	a/t	t	W
3912442	12753	4	94756853	g/t	t	K
2082553	15585	4	94759685	a/g	c	Y
6831638	17299	4	94761399	c/t	c	Y
5860329	18816	4	94762916	-/g	g	N
2870702	24022	4	94768122	c/t	t	Y
2870703	24994	4	94769094	c/t	t	Y
1948016	26637	4	94770737	g/t	t	K
6835836	27635	4	94771735	c/g	g	S
1994253	28773	4	94772873	t/c	t	Y
1905710	29430	4	94773530	a/t	a	W
1485019	29876	4	94773976	t/a	t	W
978191	30364	4	94774464	c/t	a	R
1385405	31057	4	94775157	c/a	t	K
7694361	31782	4	94775882	c/t	t	Y
1905711	33400	4	94777500	a/c	c	M
1905734	35588	4	94779688	a/c	t	K
1485012	37663	4	94781763	c/g	c	S
1485013	37865	4	94781965	t/c	c	Y
4692981	38218	4	94782318	a/t	a	W
7670552	39375	4	94783475	t/c	c	Y
7670932	39559	4	94783659	c/t	c	Y
7688091	39833	4	94783933	a/g	g	R
7440540	40135	4	94784235	a/g	g	R
2171000	41698	4	94785798	g/a	t	Y
2870704	42249	4	94786349	t/c	t	Y
7655758	42571	4	94786671	g/c	c	S
7661436	42977	4	94787077	g/a	g	R
7662289	43548	4	94787648	t/c	c	Y
7667044	43631	4	94787731	g/a	a	R

dbSNP	Position in SEQ ID NO:3	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
7691929	43705	4	94787805	t/c	t	Y
5860330	43817	4	94787917	tt/t-	t-	N
901013	44374	4	94788474	a/c	g	K
901012	44464	4	94788564	a/c	g	K
901011	44788	4	94788888	t/c	g	R
1948018	48962	4	94793062	a/g	c	Y
2870705	48993	4	94793093	t/g	t	K
1948017	49110	4	94793210	c/t	a	R
1905733	49434	4	94793534	c/t	a	R
1385408	49523	4	94793623	t/g	t	K
1385409	49742	4	94793842	c/g	c	S
1385410	49907	4	94794007	g/c	g	S
1485026	50028	4	94794128	g/c	g	S
1485027	50089	4	94794189	g/t	t	K
2904483	51588	4	94795688	c/a	a	M
1385406	52899	4	94796999	c/a	a	M
1905732	54088	4	94798188	a/c	t	K
2046418	56538	4	94800638	c/t	a	R
2200377	59071	4	94803171	t/c	g	R
1905731	59110	4	94803210	a/c	t	K
1905730	59178	4	94803278	a/g	c	Y
975713	61087	4	94805187	t/c	a	R
6820985	61300	4	94805400	c/g	c	S
7670441	62171	4	94806271	t/c	c	Y
6810794	62783	4	94806883	t/a	t	W
7676623	62983	4	94807083	t/c	c	Y
1154861	63908	4	94808008	c/t	t	Y
1032125	64088	4	94808188	g/t	g	K
1485022	64941	4	94809041	a/t	a	W
1485024	65050	4	94809150	a/g	g	R
3913651	68953	4	94813053	c/t	c	Y
4693319	70093	4	94814193	t/c	c	Y
1872383	71308	4	94815408	c/a	g	K
2200376	73009	4	94817109	t/a	t	W
7668090	74002	4	94818102	a/g	g	R
7692930	74294	4	94818394	t/c	t	Y
967096	74879	4	94818979	g/c	g	S
6822249	76936	4	94821036	g/t	t	K
6532405	77195	4	94821295	g/a	g	R
1017897	77683	4	94821783	t/c	c	Y
7672674	78283	4	94822383	t/c	t	Y
7694568	78331	4	94822431	c/t	c	Y
2904484	79362	4	94823462	g/c	c	S
7340830	80357	4	94824457	c/t	c	Y
1485033	80653	4	94824753	t/c	g	R
2870706	80840	4	94824940	a/g	g	R
1905729	83203	4	94827303	a/g	c	Y

dbSNP	Position in SEQ ID NO:3	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
4693320	85405	4	94829505	t/c	t	Y
6848749	86441	4	94830541	g/t	t	K
6532406	86967	4	94831067	g/a	g	R
6532407	87121	4	94831221	t/c	t	Y
1905728	89617	4	94833717	t/c	g	R
6819866	90969	4	94835069	t/a	a	W
1905727	94249	4	94838349	g/c	g	S
7674069	95811	4	94839911	t/g	t	K
1905724	96690	4	94840790	t/g	c	M
1905723	96731	4	94840831	a/g	c	Y
1485020	97267	4	94841367	c/g	c	S
6814101	97414	4	94841514	t/g	g	K

Assay for Verifying and Allelotyping SNPs

[0241] The methods used to verify and allelotype the proximal SNPs of Table 16 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 17 and Table 18, respectively.

TABLE 17

DbSNP rs#	Forward PCR primer	Reverse PCR primer
1433661	CTCCCAAAGCCATGGGATTC	CAGGAATGCAATCTAAGGCC
1485009	CCTAACCCTCTTCTGGAAAA	GACCTATAAAGACAGGAATGC
7681947	ACCAGAACAGGACGAATTAG	CCCCAATGTTATGCATACTC
1816432	GTTCTAGCCTTACTGAGATAG	GCTTAAAGACCTTCAAACTAC
1485018	AAAGATCGCTTGAAACTGGG	TTGCTCTGTTGTCAAGGCTG
1485017	TGACCAGGCTGGTCTAAAAC	TAATCCCAGCAGTTTGGGAG
7438397	GAGATCTAGCAATCCTCCTG	GAAACCTAATTTTGAGCCTG
6834311	TCATTGTGTTACAGATGCTC	CCAGGCTCAAAATTAGGTTTC
1368717	AAATCTGCAGACAAGGACAG	AGCAACCTGGTTTTAAGAGC
1017391	ATGCACAAATGACAGTAGGG	GGACATTGTAGTAGTCAGGG
2870701	ATGCACAAATGACAGTAGGG	GGACATTGTAGTAGTCAGGG
7679839	CCACAGGAACTATTTGCTGG	GGAACAGCACAAGCAAAAAC
1385404	CCCAGCATCCTTGCTAATAC	CCTGTCAAGATGCATAGGAG
1368716	TGGGAGAACTGGGAAAATGG	TGCTACATACTGTAGCAGCC
4693316	ATCCTGTACTGCACTTCAGC	ACCCTCCATGGGAGTTTTTC
1905707	TTTTCTGTAGTTTCCCGAGC	AATGCAATCCACAGAGCAAG
1905708	GGAACAAATGAAGATAGATG	GGACCTATGTTTTATGAGTTC
1905709	TAGAATAGTTTCTCAGTGTC	GAACTCATAAAACATAGGTCC
3912442	CATAACTCAGGCAAAACAAC	GTTGAAAAAGCTCTATATTGG
2082553	TGGAGCCCACTCAGATATTC	CCCTGGAACATGTAAATGTG
6831638	CCATATACTTACCTTTACTG	AATGTGAGTTCTGAAGATGG
5860329	CCATGCTGGCTTTTGTGAAC	TCCTGACCCTTAGTCCAATG
2870702	CAAAGAATGAGAGGGAAGGC	TGTGGCCTACCAGAATTTGC
2870703	AATAAAGAGGACAGCAGGAG	CACAGTTGTACTCCTAGCTC
1948016	GAATGAATGCAGTGTGCCAG	TGAACCTATCTTGCAGGTGG

DbSNP rs#	Forward PCR primer	Reverse PCR primer
6835836	TTATTTAGGAAGCACTCAGC	GAGTCCAAGACTAGTAAAGAG
1994253	TTCACCTTTCTGTGTTCTCC	AAGAAGAAGAACAACTGTAC
1905710	CATTAAGTGAAGAGCTATTCC	CTTAAAAGCTATTCGTTAAGC
1485019	TTTAAGCTCCCCAAAAGGTG	TACTGTGACATTCCCTTCTC
978191	AGGTCTTATCTCTTGGAATG	CCCATTCAGATATATAGACAG
1385405	CATTCAGACCTGTGACAGGA	TGATGGGACTGTTCTTCAGG
7694361	CTAGTGATTCTGTGAATGTTG	CAATGTGATTGTGTTGGAAG
1905711	CAGTTAACACTCCATATCCAC	GAGCCTCAGTTTGTTGAATC
1905734	AATTTCAAGATCAGAGATCC	CCTAACATGAGTCCATTCTG
1485012	ATACACCTATGCTAAGAAGC	GCAGAAAAACTAGTACGATAC
1485013	ACACATCATAGTTTTACTGC	GGATGAAACTATGTGAAAGC
4692981	GCTAGCCTGGAATCAAACAC	CCGAAAACAAAAGCACCCTG
7670552	TTTGGCTGCAATCAACCATG	ATCCCAAGAGAAATGAAAAC
7670932	CCTTGAAAGCATGCTAAGGG	TGAGTCCCTATAGATTTGTC
7688091	TGGATTGTGCACTTTGGATG	ACCAGACTCTCTCTTTTTTA
7440540	TGCACAGACTGTTAAGGGAG	TCATCCTTTAAGCCTTGCCG
2171000	TGAGCCAAGTATGGAAAAAC	CTGGGATAATTGTTGGGATC
2870704	ATGCTCTTGGAGCTCAGAAG	ACATATTTCCCTGACCTCCC
7655758	GTCAAAACCTCTTACTGTGC	CATGGCAGCCTTACCAAAGA
7661436	ACTGAAATTCCTTGCTGAAC	TCTCCAGGTAAACTTCCAAG
7662289	AAAACAGTGAAAGACCTTGC	TATTGAGGCCGAGTAGTCTG
7667044	ACTGATAAAACAAAGGAGGG	GAAAGCAAGGTCTTTCACTG
7691929	TTTCTCAGCATATGTTCCAG	ATCAGTCTCAGGGAAGATGC
5860330	CTGCCTAGATGAAACAAATG	CTGCTGGAACATATGCTGAG
901013	AAGGTGCATCCCTCAGAAAG	GCCTGGCTGATCTAAGGTTT
901012	CTGAAATAGTGAGGGTAGTG	AAGATGTGGAGAGAACAAGG
901011	AAAAGGGAAGGGAAGTGTGG	ACTCTCCTCCAAGAGCTTTC
1948018	TTTATCAGCAAGCATGTGAG	GTTTTCTGGAAAACTTTTACC
2870705	TAGTGCATGAGAAAGTCTAC	TTGCTTATCTGACTGGCAAG
1948017	GTTTAACAGCAACCATTGAGG	CCCCAAAGGTATGTTAAGAG
1905733	TGGAAGAGTATTAACTTCTG	TTAGTCTATTGATTGAAAGC
1385408	ACTCTTCCAAAGACTGATGC	GGATGTGGGGTTATAAGAAC
1385409	TTGCCTCTTCCAGTCTATTG	CAGAGGAGAGACATGATCAG
1385410	AGAGACAGAAGAGACACTAG	CCCCATCCAAATACATTGCC
1485026	GAGAAGAAGGCATATGTGAG	CTCACTCTTTCTTCTGTCTG
1485027	TGGTGATGATAGGTGCTGTG	ACACAGGTGCTTTCTAATTG
2904483	CTTCTCTCCATGAAACTCAG	AGGAGATATCAAGACACCAG
1385406	CTCCATTATCTGTTTGCAGT	CTCTGAGTAGTGTTTTCTGG
1905732	CTGTTCCTTGTTTCTTCTGC	GTGGTGTTTGCCATTAAAAG
2046418	AGGAGAGAAGTCTATGTCCC	TTCTTACATTCTAGAGCCTT
2200377	GGATGGACTATTGTTTTCTT	CCTATCTTGATTAGATTACAG
1905731	TCACTGCTTAGAAACTAATC	AAACACCTTTCTGAGAATTG
1905730	AAACTGAAGCAGATTCTGAC	CTTTGTTCTTTCAATTCTCAG
975713	GCTAATTTTCCTAGGATCTC	AGTGAGCCCCAAAATCTGTG
6820985	CTAAGCCTAATAAAGGAGGG	ATATATAGAAAAGACTGGAG
7670441	CAATAATGACATGCTCTTAC	CGTATAGCAATCATACACAAG
6810794	ATGATGGAGCTCCAGAAAAG	CAGAGATATTTAGTGGCAAC
7676623	TTCAGAGCTCACTTTCCAAC	GGGGCTACCAAACTTAAAAG
1154861	GACACTGAATCCATAGATCG	CCTTTTTAAATAACCAGTGAC

DbSNP rs#	Forward PCR primer	Reverse PCR primer
1032125	CCTCATGCTTTAATAGGTAG	CACACATTTCTATAGTACTTG
1485022	AAATTAGGCTGAAGGAACAG	GCTTGATGGTTATTACAATGG
1485024	CGTTTCCACCACCTGGAAAT	CTGGCTTTCTTCCATTGTTG
3913651	CCTTGAGTATCTAAGAAAGGC	AAAATATACTTGTTTTGAGC
4693319	TCAAGAGTGGAAAGGGAGAG	CAGCTCCACTCACTATCTTG
1872383	TGGCCTCAAATGCATGTGTC	CTTTGGAGCTATCCAAATGG
2200376	GTGTTTGAGAAAGAAGTGTG	GAGTTCAGACAGAGTATGAG
7668090	GTAGGCCTGAGAATGCATTG	GACATTCCATTATTCCTCCC
7692930	ATGAGAACACATGGACACGG	TCTCCTAATGCTATCCCTCC
967096	GATTGGAAAAGGGCAACAGG	TCCATGAGAATGTTCCTCAG
6822249	AAAGGATGTTTCCATTTCTC	CCTAGAAAGGTAGTTGATGC
6532405	CTCAGGATCATTGAGACTTAG	CTTGAAAGATAACTGCGGAC
1017897	TGCCCAAACTGCAAATACAC	GCTACATTAAGTGAATTCTAC
7672674	CTACAACAGACAAGGATGGG	CAATGCCTTCAGCATTTTCC
7694568	CCATTTCTAATGGGTACCTC	TGCCATACACTACAACAGAC
2904484	ATTTTCACATTGCTTTGCCC	GAACAAGCAGAGCAAGTAAG
7340830	CAATCTCAAAACAGTGTTTAC	ACTAAGAAAGGAGAGAACAG
1485033	CTGGCTGGAATGTTAATAGG	TGTGCTCCTCAGTACATAGC
2870706	CCTGCAGGAAGAAAATAGGC	AGGGAAGCAAAACTAAGATG
1905729	GGGAATTACTCTAGCTCTTC	AGGAAAGAGTAGGCCAAATG
4693320	TCAAACTAGTAGCCCACAAA	GTATAGATTACAGTAGATGTG
6848749	CCATCTTTTTGTCTGCATTC	CAGTCAATTTTCATATTGCC
6532406	GCATGGCTCTTAGGAGAAAG	GCTGGAAGGGAAAATGGTAC
6532407	TTGTTTTCCTAGGCTCCAGC	TGGCTACAATAGGGAGACTG
1905728	GCAAGTTCATTTTCATATAGCG	TAAAACAGTGTGAAATTTTG
6819866	CACACATAGCTCTTCTGTAAG	GCCTCGAGGAAAAAAAAATAG
1905727	AAGGGATAACAAGACAAATG	ACCCCATGATCTACTATTTC
7674069	CAGTTACTCCAAACTTACGG	GGTAACAAAGGCACTCAAGG
1905724	GCTCTCAGTGTCTTTTAATG	CGCATATGATTAGCTACTTC
1905723	GGGTTTATAAGCCTTTTCTTC	CTTGCTAACAATGAAAAGGTG
1485020	GTTGTCATTTTATGTCCTCCG	TAGAGTCACTGCCAATAAAC
6814101	GAGGACTTCAATGACTTTGC	TCAGACACAAACATCTGAAG

TABLE 18

dbSNP rs#	Extend Primer	Term Mix
1433661	CATGGGATTCCAGGTGT	ACT
1485009	CCTCTTCTGGAAAATCTTAA	ACT
7681947	ACGAATTAGTCCAAGGA	ACT
1816432	TGAGATAGTTAATTTTGTTTTCCA	ACT
1485018	CAGTGAGCCATGACTGT	ACT
1485017	GGTCTAAAACTCCTGAGAT	CGT
7438397	CTCCTGCCTTGGCCTCC	ACG
6834311	GAAGAGAAAGAGATGGC	ACG
1368717	AGGGATCGGAAATTTAAGAAGAA	ACT
1017391	AATGACAGTAGGGATTATAGTAA	ACT
2870701	GACAGTAGGGATTATAGTAAG	CGT
7679839	ATTTGCTGGCCCTTTAA	ACT

dbSNP rs#	Extend Primer	Term Mix
1385404	CCTAAAGCAGCCACTGT	ACT
1368716	CAATGAGAAATGCCAGC	ACG
4693316	GCGATAGAGTGAGATTCTG	ACG
1905707	TGTAGTTTCCCGAGCTAGATT	ACT
1905708	AGATAGATGAATGGAGAACCC	ACT
1905709	GTTTCTCAGTGTCATCAAATA	CGT
3912442	AAAAAGACATATCTTCTTTTAGTG	CGT
2082553	CCACTCAGATATTCCATAAC	ACT
6831638	CCTTTACTGTGATATTTATTTCTT	ACG
5860329	GCTCCTGAAACATATTCATG	ACT
2870702	GAAGGCATTATGACATGAAT	ACG
2870703	TTGTTTATTCAAATCTGCCA	ACG
1948016	GCCAGTAATCTCTCCAATGAT	CGT
6835836	CACTCAGCTGAATAGACA	ACT
1994253	CTGACTCATACATCCTTTG	ACT
1905710	GAGCTATTCCAATGTGCT	CGT
1485019	AAGCTCCCCAAAAGGTGTTTAATT	CGT
978191	TGTACAAATCTGAGGGC	ACG
1385405	CCTGTGACAGGATTCCAGCA	CGT
7694361	GGATTACAGTAGTTTCCC	ACG
1905711	CCATATCCACAGGTTCT	ACT
1905734	CTTAGCCACTCTGATAATCT	ACT
1485012	ACACTGCAAAAAGCACT	ACT
1485013	TGCAGAGATAATGTATGTAGAA	ACT
4692981	TCAAACACAGTTTATATGAGATAA	CGT
7670552	CAACCATGCTGCTATGA	ACT
7670932	CATGCTAAGGGAAAGAAG	ACG
7688091	GGATGGGTGAATTGTATATTAT	ACT
7440540	TTAAGGGAGAGCATGAAA	ACT
2171000	GGTAGAAATGGACTTTGA	ACG
2870704	GCAGCTTCCTAACAAAAA	ACT
7655758	GCCTTTGAAAGAATCCAA	ACT
7661436	CTTTTTTGTTTCTATCCAGG	ACG
7662289	TGCTTCTTTATTCCCCA	ACT
7667044	GCTTTGTTTTTGATGAGTG	ACG
7691929	TTAATTCCTGAGACGTGT	ACT
5860330	CTTGCAAGTGATTAAAAAAAAAA	ACT
901013	GAAAGCATTCATCTCACTA	ACT
901012	GTAGTGTCTACAAAGGGTATA	ACT
901011	GTTGATCTCCTTCCTGG	ACT
1948018	GAGAAAGTAGACTTTCTCAT	ACT
2870705	CTTTCTCACATGCTTGC	ACT
1948017	CAGCAACCATTGAGGGTGAAT	ACG
1905733	TGTCCTTGACTGATTTTTAG	ACG
1385408	TCTAGGAAGTATGAGATGG	ACT
1385409	CAATAGTAACTGTCAACTGT	ACT
1385410	CAAGGAAGCTAGAGCCA	ACT
1485026	CTTCAATTAGAAAGCACCT	ACT

dbSNP rs#	Extend Primer	Term Mix
1485027	GTGCTGTGAAGAGAAATTA	CGT
2904483	GGATTGTTTCTTCCTCT	CGT
1385406	TGTATACAGAAAAAAGCATGA	CGT
1905732	GTTGGTGCAAAAGTAACT	ACT
2046418	GTCTATGTCCCTGACAC	ACG
2200377	AAAAATTAAAACATTCACTGCTTA	ACT
1905731	TTCTGTAATCTAATCAAGATAGG	ACT
1905730	TCTGACAGGACTAAAGAA	ACT
975713	GGTCAGCTAAGGATTTTAGA	ACT
6820985	AAAGGAGGGCTCTACCC	ACT
7670441	CATGCTCTTACATGCAAATA	ACT
6810794	CCAGAAAAGCAAGCTTATTA	CGT
7676623	TCTTCTTTAATGTGATGGTAC	ACT
1154861	GAATCCATAGATCGTATACTAAT	ACG
1032125	ATTTATATTCCGCCCCA	CGT
1485022	TCCAAAACGACCAGTCA	CGT
1485024	TTCATGCTAACTGATTATCAAAT	ACT
3913651	GGCAAAATTCCATGGCC	ACG
4693319	GAGTAATTGGACCTCTAC	ACT
1872383	CCATTCCATATCCTTACC	CGT
2200376	TTTTACATCTCACCCCA	CGT
7668090	GAATGCATTGGAGTGAG	ACT
7692930	ATCACACTCCGGGGACT	ACT
967096	AGGGCAACAGGGACACA	ACT
6822249	GTATTCACCATTGCAAAAA	CGT
6532405	GAACAACTGAAATCTGAAGTA	ACG
1017897	AACTGCAAATACACATTTCA	ACT
7672674	GTTTTGAACACTTAATGTTTG	ACT
7694568	GATTTCACCCCTTTCCC	ACG
2904484	AAATGTTCTCCAAGAAAGAT	ACT
7340830	GTTTACATATGAGGAAATGTAG	ACG
1485033	GCCCTCTAAAGACATGA	ACT
2870706	GAAGAAAATAGGCTGATTTTAT	ACT
1905729	CTAGCTCTTCAGAATTAATTGG	ACT
4693320	CCCACAAAGTCTTATGCA	ACT
6848749	CCAACAGAGAGAGGTATTTA	CGT
6532406	CATGGTCTACACACCTTTA	ACG
6532407	CCTTGTAAGACTACCTGAA	ACT
1905728	CAGTTAATAATTGTAGATCCATG	ACT
6819866	GCTCTTCTGTAAGAAGTCT	CGT
1905727	ATGATTGTAGATCATTTTGATGTA	ACT
7674069	TTCCTAACTCTTCACCTT	ACT
1905724	CATTGTTAGCAAGTGGAA	ACT
1905723	GAGCATAAAGATGCTCTCAGT	ACT
1485020	CTCCGTTATCTCCATGT	ACT
6814101	GCAAATGTAGTTGTATGTAATTT	ACT

Genetic Analysis

[0242] Allelotyping results are shown for female cases and controls in Table 19. The allele frequency for the A2 allele is noted in the fifth and sixth columns for control pools and case pools, respectively, where "AF" is allele frequency. Some SNPs do not have an allele frequency disclosed because of failed assays.

TABLE 19

dbSNP rs#	Position in SEQ ID NO:3	Chromosome Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
1433661	206	94744306	T/C	T=0.11	T=0.17	0.0089	1.64	T
				C=0.89	C=0.83			
1485009	243	94744343	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
7681947	2467	94746567	T/C	T=	T=0.89			
				C=	C=0.11			
1816432	3550	94747650	A/G	A=0.56	A=0.61	0.0779	1.23	A
				G=0.44	G=0.39			
1485018	4994	94749094	C/G	C=1.00	C=0.99			
				G=0.00	G=0.01			
1485017	5167	94749267	G/T	G=	G=			
				T=	T=			
7438397	5193	94749293	C/T	C=0.94	C=0.94	0.8804	0.96	C
				T=0.06	T=0.06			
6834311	5273	94749373	G/A	G=0.51	G=0.56	0.1212	1.20	G
				A=0.49	A=0.44			
1368717	5733	94749833	A/G	A=0.10	A=0.13	0.1573	1.31	A
				G=0.90	G=0.87			
1017391	7817	94751917	A/C	A=0.64	A=0.63	0.6402	0.94	C
				C=0.36	C=0.37			
2870701	7818	94751918	T/A	T=0.00	T=0.00			
				A=1.00	A=1.00			
7679839	8612	94752712	T/G	T=0.97	T=0.90	0.0000	0.31	G
				G=0.03	G=0.10			
1385404	9158	94753258	A/G	A=	A=1.00			
				G=	G=0.00			
1368716	9285	94753385	G/A	G=0.89	G=0.83	0.0251	0.62	A
				A=0.11	A=0.17			
4693316	10680	94754780	G/A	G=0.57	G=0.53	0.2431	0.87	A
				A=0.43	A=0.47			
1905707	11866	94755966	T/C	T=0.07	T=0.13	0.0008	1.99	T
				C=0.93	C=0.87			
1905708	11958	94756058	A/G	A=0.97	A=0.92	0.0003	0.35	G
				G=0.03	G=0.08			
1905709	12044	94756144	A/T	A=0.05	A=0.06	0.8882	1.08	A
				T=0.95	T=0.94			
3912442	12753	94756853	G/T	G=0.00	G=0.00			
				T=1.00	T=1.00			

dbSNP rs#	Position in SEQ ID NO:3	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
2082553	15585	94759685	A/G	A=0.00	A=0.11			
				G=1.00	G=0.89			
6831638	17299	94761399	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
5860329	18816	94762916	-/G	-=0.00	-=0.00			
				G=1.00	G=1.00			
2870702	24022	94768122	C/T	C=0.66	C=0.65	0.8596	0.98	T
				T=0.34	T=0.35			
2870703	24994	94769094	C/T	C=0.63	C=0.62	0.7613	0.96	T
				T=0.37	T=0.38			
1948016	26637	94770737	G/T	G=0.52	G=0.47	0.1367	0.84	T
				T=0.48	T=0.53			
6835836	27635	94771735	C/G	C=0.00	C=0.00			
				G=1.00	G=1.00			
1994253	28773	94772873	T/C	T=0.92	T=0.87	0.0134	0.61	C
				C=0.08	C=0.13			
1905710	29430	94773530	A/T	A=0.39	A=0.34	0.2784	0.83	T
				T=0.61	T=0.66			
1485019	29876	94773976	T/A	T=0.14	T=0.19	0.0338	1.42	T
				A=0.86	A=0.81			
978191	30364	94774464	C/T	C=0.00	C=0.00			
				T=1.00	T=1.00			
1385405	31057	94775157	C/A	C=0.68	C=0.66	0.6519	0.94	A
				A=0.32	A=0.34			
7694361	31782	94775882	C/T	C=0.21	C=0.24	0.1629	1.22	C
				T=0.79	T=0.76			
1905711	33400	94777500	A/C	A=0.00	A=0.00			
				C=1.00	C=1.00			
1905734	35588	94779688	A/C	A=0.92	A=0.88	0.0229	0.61	C
				C=0.08	C=0.12			
1485012	37663	94781763	C/G	C=0.96	C=0.91	0.0032	0.47	G
				G=0.04	G=0.09			
1485013	37865	94781965	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
4692981	38218	94782318	A/T	A=0.68	A=0.72	0.1741	1.19	A
				T=0.32	T=0.28			
7670552	39375	94783475	T/C	T=0.11	T=0.22	0.0000	2.26	T
				C=0.89	C=0.78			
7670932	39559	94783659	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
7688091	39833	94783933	A/G	A=0.00	A=0.13			
				G=1.00	G=0.87			
7440540	40135	94784235	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
2171000	41698	94785798	G/A	G=0.00	G=0.00			
				A=1.00	A=1.00			
2870704	42249	94786349	T/C	T=0.53	T=0.53	0.8418	1.02	T
				C=0.47	C=0.47			
7655758	42571	94786671	G/C	G=0.21	G=0.25	0.0531	1.32	G

dbSNP rs#	Position in SEQ ID NO:3	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
				C=0.79	C=0.75			
7661436	42977	94787077	G/A	G=0.44	G=0.45	0.7243	1.04	G
				A=0.56	A=0.55			
7662289	43548	94787648	T/C	T=0.24	T=0.29	0.1625	1.27	T
				C=0.76	C=0.71			
7667044	43631	94787731	G/A	G=0.18	G=0.22	0.0669	1.31	G
				A=0.82	A=0.78			
7691929	43705	94787805	T/C	T=0.93	T=0.90	0.0439	0.64	C
				C=0.07	C=0.10			
5860330	43817	94787917	T/-	T=0.48	T=0.52	0.1449	1.19	T
				-=0.52	-=0.48			
901013	44374	94788474	A/C	A=0.00	A=0.00			
				C=1.00	C=1.00			
901012	44464	94788564	A/C	A=0.00	A=0.00			
				C=1.00	C=1.00			
901011	44788	94788888	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
1948018	48962	94793062	A/G	A=0.10	A=0.19	0.0002	2.20	A
				G=0.90	G=0.81			
2870705	48993	94793093	T/G	T=1.00	T=1.00			
				G=0.00	G=0.00			
1948017	49110	94793210	C/T	C=0.12	C=0.17	0.0068	1.60	C
				T=0.88	T=0.83			
1905733	49434	94793534	C/T	C=0.02	C=0.03	0.5881	1.41	C
				T=0.98	T=0.97			
1385408	49523	94793623	T/G	T=1.00	T=1.00			
				G=0.00	G=0.00			
1385409	49742	94793842	C/G	C=1.00	C=1.00			
				G=0.00	G=0.00			
1385410	49907	94794007	G/C	G=0.53	G=0.51	0.5313	0.93	C
				C=0.47	C=0.49			
1485026	50028	94794128	G/C	G=1.00	G=1.00			
				C=0.00	C=0.00			
1485027	50089	94794189	G/T	G=0.00	G=0.00			
				T=1.00	T=1.00			
2904483	51588	94795688	C/A	C=0.68	C=0.65	0.4125	0.90	A
				A=0.32	A=0.35			
1385406	52899	94796999	C/A	C=0.00	C=0.00			
				A=1.00	A=1.00			
1905732	54088	94798188	A/C	A=0.48	A=0.47	0.8078	0.97	C
				C=0.52	C=0.53			
2046418	56538	94800638	C/T	C=0.64	C=0.65	0.9229	1.01	C
				T=0.36	T=0.35			
2200377	59071	94803171	T/C	T=0.00	T=0.04			
				C=1.00	C=0.96			
1905731	59110	94803210	A/C	A=1.00	A=1.00			
				C=0.00	C=0.00			
1905730	59178	94803278	A/G	A=0.74	A=0.73	0.9270	0.99	G
				G=0.26	G=0.27			

dbSNP rs#	Position in SEQ ID NO:3	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
975713	61087	94805187	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
6820985	61300	94805400	C/G	C=0.99	C=0.99			
				G=0.01	G=0.01			
7670441	62171	94806271	T/C	T=0.67	T=0.66	0.7167	0.96	C
				C=0.33	C=0.34			
6810794	62783	94806883	T/A	T=1.00	T=1.00			
				A=0.00	A=0.00			
7676623	62983	94807083	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
1154861	63908	94808008	C/T	C=0.73	C=0.70	0.2406	0.84	T
				T=0.27	T=0.30			
1032125	64088	94808188	G/T	G=0.50	G=0.51	0.9342	1.01	G
				T=0.50	T=0.49			
1485022	64941	94809041	A/T	A=1.00	A=0.98			
				T=0.00	T=0.02			
1485024	65050	94809150	A/G	A=0.04	A=0.12	0.0000	3.18	A
				G=0.96	G=0.88			
3913651	68953	94813053	C/T	C=0.40	C=0.41	0.6884	1.05	C
				T=0.60	T=0.59			
4693319	70093	94814193	T/C	T=0.81	T=0.80	0.7260	0.95	C
				C=0.19	C=0.20			
1872383	71308	94815408	C/A	C=0.35	C=0.38	0.4769	1.10	C
				A=0.65	A=0.62			
2200376	73009	94817109	T/A	T=0.65	T=0.63	0.6117	0.92	A
				A=0.35	A=0.37			
7668090	74002	94818102	A/G	A=0.61	A=0.59	0.4762	0.92	G
				G=0.39	G=0.41			
7692930	74294	94818394	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
967096	74879	94818979	G/C	G=0.00	G=0.00			
				C=1.00	C=1.00			
6822249	76936	94821036	G/T	G=0.20	G=			
				T=0.80	T=			
6532405	77195	94821295	G/A	G=0.44	G=0.46	0.5246	1.08	G
				A=0.56	A=0.54			
1017897	77683	94821783	T/C	T=0.75	T=0.72	0.3873	0.89	C
				C=0.25	C=0.28			
7672674	78283	94822383	T/C	T=0.81	T=0.82	0.6661	1.07	T
				C=0.19	C=0.18			
7694568	78331	94822431	C/T	C=0.82	C=0.91	0.0004	2.33	C
				T=0.18	T=0.09			
2904484	79362	94823462	G/C	G=0.67	G=0.66	0.8007	0.97	C
				C=0.33	C=0.34			
7340830	80357	94824457	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
1485033	80653	94824753	T/C	T=0.74	T=0.73	0.6809	0.95	C
				C=0.26	C=0.27			
2870706	80840	94824940	A/G	A=0.65	A=0.65	0.9576	1.01	A

dbSNP rs#	Position in SEQ ID NO:3	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
				G=0.35	G=0.35			
1905729	83203	94827303	A/G	A=0.66	A=0.62	0.1186	0.82	G
				G=0.34	G=0.38			
4693320	85405	94829505	T/C	T=0.67	T=0.73	**0.0335**	1.32	T
				C=0.33	C=0.27			
6848749	86441	94830541	G/T	G=0.14	G=0.22	**0.0016**	1.68	G
				T=0.86	T=0.78			
6532406	86967	94831067	G/A	G=0.98	G=0.95	**0.0460**	0.44	A
				A=0.02	A=0.05			
6532407	87121	94831221	T/C	T=0.95	T=0.91	**0.0301**	0.57	C
				C=0.05	C=0.09			
1905728	89617	94833717	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
6819866	90969	94835069	T/A	T=0.51	T=0.42	**0.0157**	0.70	A
				A=0.49	A=0.58			
1905727	94249	94838349	G/C	G=0.06	G=0.09	0.1689	1.48	G
				C=0.94	C=0.91			
7674069	95811	94839911	T/G	T=1.00	T=1.00			
				G=0.00	G=0.00			
1905724	96690	94840790	T/G	T=0.06	T=0.07	0.3032	1.31	T
				G=0.94	G=0.93			
1905723	96731	94840831	A/G	A=0.71	A=0.70	0.7768	0.96	G
				G=0.29	G=0.30			
1485020	97267	94841367	C/G	C=1.00	C=1.00			C
				G=0.00	G=0.00			
6814101	97414	94841514	T/G	T=	T=0.00			G
				G=	G=1.00			

[0243] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 3. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 3 can be determined by consulting Table 19. For example, the left-most X on the left graph is at position 44917643. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0244] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression

models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0245] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is place at the 3' end of each gene to show the direction of transcription.

Example 8

PDE4D Proximal SNPs

[0246] It has been discovered that a polymorphic variation (rs1498608) in a gene encoding *PDE4D* is associated with the occurrence of low BMD (see Examples 1 and 2). One hundred sixteen additional allelic variants proximal to rs1498608 were identified and subsequently allelotyped in low BMD case and high BMD control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 20. The chromosome position provided in column four of Table 20 is based on Genome "Build 34" of NCBI's GenBank.

TABLE 20

dbSNP	Position in SEQ ID NO:4	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
6886495	249	5	58309549	g/c	g	S
6450498	543	5	58309843	a/t	a	W
1472456	973	5	58310273	g/a	c	Y
4700315	1076	5	58310376	a/g	a	R
4700316	1276	5	58310576	g/c	g	S
7714708	1599	5	58310899	a/g	a	R
7710479	2755	5	58312055	c/t	c	Y
2968013	2911	5	58312211	g/c	c	S
2968014	4466	5	58313766	a/g	a	R
2968015	5754	5	58315054	t/c	c	Y
1391648	5762	5	58315062	a/g	c	Y
2055297	5967	5	58315267	a/g	c	Y
2055296	5972	5	58315272	a/g	t	Y
3989138	6390	5	58315690	-/aa	a	N
4700317	6984	5	58316284	c/t	t	Y
2036220	7234	5	58316534	g/a	c	Y
7727206	8198	5	58317496	g/t	t	K
7723432	8369	5	58317669	a/g	a	R
1546221	9585	5	58318885	c/t	g	R
4479801	11084	5	58320384	c/t	c	Y

dbSNP	Position in SEQ ID NO:4	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
4395595	11153	5	58320453	c/t	c	Y
4395596	11187	5	58320487	t/c	c	Y
4699932	11290	5	58320590	g/a	a	R
2936201	11386	5	58320686	a/g	t	Y
7356672	11441	5	58320741	c/t	c	Y
2936200	12373	5	58321673	a/g	c	Y
1909296	12602	5	58321902	c/a	g	K
7703131	13763	5	58323063	c/g	c	S
7445308	18697	5	58327997	a/t	t	W
3087748	18854	5	58328154	t/c	g	R
4321723	19107	5	58328407	c/t	g	R
2968016	19310	5	58328610	c/t	c	Y
5868151	20074	5	58329374	c/-	c	N
1874858	20145	5	58329445	t/c	g	R
1874857	20281	5	58329581	a/c	t	K
7712922	23117	5	58332417	c/t	t	Y
4631140	23585	5	58332885	g/a	a	R
4469166	23906	5	58333206	t/c	t	Y
1078369	24046	5	58333346	c/g	g	S
1078368	24450	5	58333750	g/a	c	Y
2968006	24619	5	58333919	a/g	t	Y
2968005	24637	5	58333937	g/t	c	M
2936190	24894	5	58334194	c/t	c	Y
2409613	25030	5	58334330	g/c	g	S
4415048	25732	5	58335032	g/c	g	S
2968004	27106	5	58336406	a/c	g	K
2968003	27395	5	58336695	g/a	t	Y
2968002	28971	5	58338271	a/g	c	Y
2936191	29755	5	58339055	t/a	t	W
1498610	30988	5	58340288	a/g	g	R
6874662	31827	5	58341127	c/a	a	M
3060393	31843	5	58341143	a/aca	a	N
7729722	32773	5	58342073	g/a	a	R
7733884	32787	5	58342087	t/c	t	Y
7714489	33099	5	58342399	t/c	c	Y
7735570	36854	5	58346154	t/c	c	Y
2936193	38026	5	58347326	g/t	g	K
2291851	38397	5	58347697	t/c	c	Y
2291852	38680	5	58347980	a/g	a	R
1498602	39626	5	58348926	t/c	t	Y
1995166	39682	5	58348982	t/c	t	Y
1498603	39710	5	58349010	g/t	t	K
1498604	39745	5	58349045	c/a	a	M
1498605	39901	5	58349201	g/a	g	R
1948651	39925	5	58349225	c/t	t	Y
4699934	40356	5	58349656	g/t	t	K
4700319	40393	5	58349693	c/t	c	Y
2279737	41230	5	58350530	a/g	a	R
7720361	41733	5	58351033	c/t	c	Y
7706419	41877	5	58351177	g/t	g	K
1006431	43555	5	58352855	t/g	g	K

dbSNP	Position in SEQ ID NO:4	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
1353747	44086	5	58353366	g/t	t	K
1498606	44134	5	58353434	c/t	c	Y
1353748	44181	5	58353481	t/g	g	K
1553113	45022	5	58354322	a/c	a	M
2968012	46856	5	58356156	t/g	c	M
2968011	48231	5	58357531	c/a	t	K
1498608	49652	5	58358952	a/t	t	W
2936189	50393	5	58359693	t/a	a	W
1498609	51103	5	58360403	c/t	t	Y
2968019	51733	5	58361033	t/c	c	Y
6891238	54733	5	58364033	t/c	t	Y
2968010	57173	5	58366473	a/t	t	W
2968009	58192	5	58367492	c/t	g	R
2936203	58506	5	58367806	g/c	g	S
1498601	59572	5	58368872	g/a	t	Y
1498600	59738	5	58369038	a/g	t	Y
1498599	61617	5	58370917	g/a	t	Y
2936202	63980	5	58373280	c/g	c	S
7730070	64161	5	58373461	c/g	g	S
6450501	66871	5	58376171	a/g	g	R
6450502	67063	5	58376363	a/t	a	W
6889456	67084	5	58376384	a/g	a	R
6894618	67477	5	58376777	c/t	t	Y
7706044	69282	5	58378582	t/c	t	Y
7707541	70363	5	58379663	a/t	a	W
7712078	70647	5	58379947	c/t	t	Y
6892860	71834	5	58381134	c/t	c	Y
6867053	72130	5	58381430	c/g	c	S
7737269	73495	5	58382795	c/t	t	Y
6864156	74542	5	58383842	t/c	t	Y
950447	75280	5	58384580	t/c	a	R
2936196	80740	5	58390040	a/g	g	R
7719347	82579	5	58391879	t/c	t	Y
1391649	82591	5	58391891	c/a	a	M
1391650	82976	5	58392276	t/c	c	Y
1391651	83040	5	58392340	g/a	a	R
1353749	85894	5	58395194	g/a	a	R
10682149	86020	5	58395320	g/gcct	g	N
5868153	86947	5	58396247	g/ag	g	N
1363882	88922	5	58398222	c/g	g	S
2409628	89662	5	58398962	t/c	t	Y
2968018	92367	5	58401667	g/a	c	Y
954740	93154	5	58402454	a/g	c	Y
986067	94979	5	58404279	t/c	c	Y
6869400	97598	5	58406898	t/c	c	Y
5010782	98532	5	58407832	t/a	a	W

Assay for Verifying and Allelotyping SNPs

[0247] The methods used to verify and allelotype the proximal SNPs of Table 20 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 21 and Table 22, respectively.

TABLE 21

DbSNP rs#	Forward PCR primer	Reverse PCR primer
6886495	AGTTTGCTTCCTGAACAATC	ATGCAGTTGAATCTCAATAC
6450498	GGACAGCTTTATGTTTAATAC	ACAAATACTCCATTGATGGT
1472456	GGGAGGTAAGGAATCATGAC	TTTTGAAGGACAAGTTCCCC
4700315	GGGAACTTGTCCTTCAAAAAG	CATTTTCTGTTACTCTGAGG
4700316	ATTGTGTCCATGCTTGGCAG	CCTCCTTCTATTGTGGAGAC
7714708	GCAACATACTAACTGGAATCC	GTTCTTCTGTTTGTCATGTGG
7710479	TGCTCCAAAAATGGCAATCG	ATTTCTACATCACCAGGGAG
2968013	CACCCCTAGATTTTGAAGGT	CAATGACACATTCCCTCACC
2968014	CCTTCCCTCCTTCATTCAAC	TGGATTCAGATTACGGGAGG
2968015	TGTCAGCTGTATGGTGAAGG	AACGCCAAGCCTATTTTCAG
1391648	AATTTGGTAAGACAGAGATG	TTGCTCTGTTCCTGAAAGAC
2055297	AAGCTTTGTTTCTCTTTCTC	AACGATTCTACATCTGCCCC
2055296	AAGCTTTGTTTCTCTTTCTC	AACGATTCTACATCTGCCCC
3989138	GCCTTCCCCCTCCTTTAAAA	CATTTCAGGATGAATACTTG
4700317	TTTTCCTGATATGATGAATG	CTAGTAATGCAGCAATAGTG
2036220	TGTATCCCAGGACATCTAGC	AACATTCAGGAAATTTCAGG
7727206	AAGGACTGGGTTTGCATTTC	CTAATCTTGGCAACATTCTG
7723432	TGAATCTAGGAGTGGATTTG	AGATCTGACCATGGTGATAG
1546221	GGAAAACTCCTTATGTTGGA	GCTGTATATTATATTACTGTG
4479801	ACTTCTAGAAGAAAACACAG	TATGCTTCATGCTTTTGTGG
4395595	AAACCACAAAAGCATGAAGC	TGGCTTGTTGTTCTGTTTTC
4395596	ATATTCCCTTCTCAAGTCTG	CTGGACTTTACCAAATTTCTC
4699932	CCATCTTTTCACTGGATTGTC	CAGACTTGAGAAGGGAATATG
2936201	GCATCTCACTGTGGTTTTAC	CACAATAAGAGGACAATCCAG
7356872	GACCATCATTAGACATTAGGG	TTAATTTGCTCTGCATCCAG
2936200	TCACAACTGGGAATTCACTG	GAGCTCCACCATTATTTCCC
1909296	CCGAGTAGCTGGGATTACAT	TGGTGAAACCCTGTCTCTAC
7703131	AGAGAAGAAGGCTTCAGACG	TTTAGCTTCTTTGCGATGGG
7445308	ATACAGCGGTTGGGACTATC	GCACATTGTGCACATGTACC
3087748	TAGTCCATAGGAATCTGCTG	GCTGCTGTACATTACAACAC
4321723	GAATTTTACTTGGAACCCTGG	CCTATCTTACTACTGAACTC
2968016	GTAAACCTTGGACTTATGGG	ACGATGTTACCCTCTTTTCC
5868151	TAGAATAGACTACATCCATC	GGATAAGGAAGTTTCTTAGG
1874858	GCCTAAGAAACTTCCTTATCC	ATATTGCCAACTAGGAGTAC
1874857	ATGAAGACTTTACTGAAGGC	CATACACTACTAACCTGTTGC
7712922	CTCTTTCTAAGGGCTTCTGG	AGAGGAGTCGGACTTTGTTC
4631140	TTGAAGTTGAGAGGGTCTCC	AAGCAAAGCACAAGCAACAG
4469166	TTAGGAGGGATGAGGAATGG	CTCCAATTGCACTGGGTTAC
1078369	TCCCTGAGCCTCTGTTTTCC	ATATGTCCCCACCACACTAC
1078368	ATGACTCATGGAGGCAACAG	GGAGAGCGATTTATGGATGG
2968006	CAGGGTTCATTTGGTGAAAC	ACTGACCTGTCTGGGATTTC
2968005	ACTGACCTGTCTGGGATTTC	CGCAGGGTTCATTTGGTGAA
2936190	AACAGACCTCCAACACAACC	TAGGATAAACTCACGGAAGG
2409613	AATAGGGTCAGTGGGATGAG	CCCACCTCCAAATCCTTTAC
4415048	CACCCAGCCTCAGAATATTT	TTCCCCACCAATACAACATC

DbSNP rs#	Forward PCR primer	Reverse PCR primer
2968004	CCCAAACATTATCTTCTGGC	GATCTCCATAAGGGTAAGTG
2968003	ACAAGAGGACAAGTGTTTAG	GGCCAGAGCTGTTCATAAAT
2968002	CCTTTGACTTTCCACAGAAC	ACACTCACTGGGTTGGGCTA
2936191	TGACCTTGATAACCTGGCTG	AGCTGTGCATATTGACTTCC
1498610	CATTAAATCACCACAGCAAC	TCCTTAGGCAGAATGGTC
6874662	ACTCTAGCCTGGGCAACAGA	ATGTCTTGTAGTTTCCAGTG
3060393	CCAGTGTATAGATCTTTCACC	AGAGCGAGACTCCATCACAA
7729722	GAGGAAAGTGTGTCTATTCAG	CCATACATCTGATAAGAGGC
7733884	TGTATCTCTGTTGGCCATTC	CCATACATCTGATAAGAGGC
7714489	AGGGTCTTGCTCTTTTGTTC	ATTGAGCCCGAGAATTTGAG
7735570	AGGATCTTCATAGAAGTGGC	AACTGAGGCTGTTTTCTCTC
2936193	CTTAGCATACAATGGGCACC	ACAGTGCCTACTATTGTCAG
2291851	GTTTCTTGCTTGTTGATGGC	TAGCCTTGGCCAAGAATTCA
2291852	ATCCTGATCTCTTACTAGTG	TTCTGTGTTCTCTCTTGAGG
1498602	CAGTAGTTTTTCAGCTAAATG	ATCCAAACATAGATCTCAAC
1995166	GCCACTTAGCAATGTGCAAG	GGGAAAGTAACTAGCTGCTC
1498603	GTGTGGTTGATTCTGTGTAAG	ACTACTGTCGAATCACCTTG
1498604	GCTTGCACATTGCTAAGTGG	CCATGGGCCTGAGTTCTTAA
1498605	CTGTGGCATTAGGCACCTTT	AGGACACTGGACATATTCAG
1948651	CAGATGAGAATAAAGGTGCC	TATTGCTCTCTTACTGGGAC
4699934	AGGACTGGTAATGTTGTGAG	GACCTTGTAAATAGGTGGCC
4700319	GACCTTGTAAATAGGTGGCC	GCTGAAGGATTCAGCCAGTA
2279737	AAAACCTCTCAATTTATTTC	ATGGCCTCCAACAAGGTAAG
7720361	ACTATGCGACTTTTCAACTG	ACCCCCTCGCCATCCGCTA
7706419	ACCCGGCCGCGGCTGATTCAT	GCCCCGCCTGCCGAGCCTT
1006431	CCTTGCCAGGTGAATTAAAG	CTATACAGAGCAGGTATTTT
1353747	TGAGAAAGTTGGAGTGCAGG	AATCATTGGTTACAATGAAG
1498608	ACTCCAACTTTCTCAAAGCC	TTGGTGAAGATGGAGGAAGG
1353748	CTCCATCTTCACCAAGTTCC	GAAATATAATGTGTGGAGCC
1553113	AGCCTTTAGGGAGTTTAGCC	GCACCAAATCTTGCTAAGTC
2968012	CAGTTCTATCTACTGTAGAC	CAGTTCCAGCTTCTCTTCTC
2968011	ATAATGGTTGCACTGACTTC	ACTGTGTGACATGGGATCTG
1498608	GAATCCCTGTTCATTCCTTG	ATAACCTCGGGGTCCAGAAA
2936189	TGATCTCAGACTTCCAGCTC	TTTGTCTTAGCTCAAGCTGC
1498609	TAGCTCAACTGTTCTTCAGG	CAGAGTGAGTGTAAATATAC
2968019	CCCAGGCCAGTATTACTGTT	TGACATTTACAGGCACTCAG
6891238	CCTTTATTCAGGCTGCAGAC	TGTGGTTTTAATGGCTGTGG
2968010	ATGTATGATTCACTCTGATG	CAAACCAATTGGTAGATTTC
2968009	GTAGGCAAAGATGAATCACG	TGTTTTACCTAGCAAGGAGC
2936203	AGTTCAGAGGTTCCAGACAG	AGCCAAGCTTGCAAACTCTG
1498601	TTGGTTAGATCCAGCTCTGC	TAAGAGGAACAGGGATCTGC
1498600	ATATAGGTACTGCTTTCTCC	CCTTGTTTCCAAATCTGAGC
1498599	GGAACATTTGGCTACATCATG	CCACAGAGCTGATTTAATTTC
2936202	TGTAAGAGGAGGGTGTGATG	TGACTCTGCAGGACTGTCTG
7730070	AAATATACTTGGGTAGAGAG	GATAGCTAACACATTTCTGAC
6450501	AGCTAGACTACATAGCCTCC	TGTAGGACATGACAGCAAAC
6450502	TAACAAGTCAAAACAGATGG	ATTTTCAATATTCTGCACAC
6889456	GGATCTCTATTAAACCTCTC	TTCAAGGGTTACTGATACTC
6894618	CCATCAACAGATGAATGGAC	TCCTTCCTTTTTTAGGGCTG
7706044	GACTTGGTATTTTGTGAGGG	TCCACCATGTGAGGACAAAG
7707541	TTTTGAGTCATGAAAAACTG	CACATGTATTAATTAAGTAGG
7712076	AAGCCATACCACAAGCATTC	GTTCACGTGAAATACACTACC
6892860	CAGAAGATGCAATGAAAAGAC	AGAACAAAATGTTAGGACGC

DbSNP rs#	Forward PCR primer	Reverse PCR primer
6867053	TTATCCGCATTCCACTCTTG	ACACTGGTCCTCATAACAAG
7737269	TTTAAAGGCTCGCACCTCAG	TCCGAAGGAAAGTGATTCTC
6864156	GTTGGATCATCAAAAAGTGG	GGCTAGAGTACTAAAAATCAG
950447	GTGAGTAGTCTGAATTTGTC	AGGCTTCAGAATCGGTCATC
2936196	CTCCAAAACATAGTAAGTGC	TGAACATTTAGAATTAGGGG
7719347	ACATTCTGAGGTCATGCAGG	CTCAGAGCCTGCATTATCTC
1391849	GCTAATGCTTTACTCTGGTC	CACACTCCAAAATGTGCAAC
1391850	TTCAGCTGATACTTGCTCCC	CTCTACCTACCAACATCGTC
1391851	TTGACGTGATAGAAGTTTGG	CTGAAAAGGGTGGCTTTCTC
1353749	TATAGAGCAAAAAGCCAAGG	CCACTTCTACCACATTCTTC
10882149	GGGAGAATATAACCATTAAGT	GAATTGCATTTTATCCAATC
5868153	CATAGGGACTATTTAAACTTC	GAGTGTTCTTTAGAGATTAAG
1363882	CTCACACAGGCAATGAGTAG	AGCCACTACTTCTCAATCTC
2409626	CCTGGTCTCAAGCAATCATC	ATAAGGCCAGATGTGGTGAC
2988018	GCAGAGAGATGAGAGGAAAC	CCTCATATCTAATCTCTCCC
954740	ATACTTGGGAGCACTCAACG	CAACAAGACGGAATCCAAAG
986067	CTACAAATTGCTTAAGCAGGG	TGATAGAGTAGAGAGACTCC
6869400	ACAAGATCGTTGAATGGTGG	GACTTTGTATACTGCCACTC
5010782	AATCTGTGGGAGTTAGTGGT	TTCCTCATCCTCATCTTCCC

TABLE 22

dbSNP rs#	Extend Primer	Term Mix
6886495	CCTGAACAATCTTAAATGC	ACT
6450498	GTCTTATGCATTTTGAAGG	CGT
1472456	GGAATCATGACTACTTGGA	ACG
4700315	GAAACAAAGCAAACGAC	ACT
4700316	TGCTTGGCAGGCTTTTT	ACT
7714708	CAGTATAAGTAATTTGGCCC	ACT
7710479	GAGCATTTTAATTGCTTCC	ACG
2988013	AGCTGAGAGCAGCCATG	ACT
2988014	GAGCTGTTTCTTTCAGTTT	ACT
2988015	ATGGTGAAGGTGGAGGA	ACT
1391848	TCAGCTGTATGGTGAAG	ACT
2055297	GTGCCTGACATAGAGTAG	ACT
2055296	GGCAGTGCCTGACATAG	ACT
3989138	CCCCTCCTTTAAAAAAAAAAA	CGT
4700317	CAAATGTAACAAAGTGCCA	ACG
2036220	GCCTCCCTGGCCTCTGC	ACG
7727206	AATCCTGTCACCTATGG	CGT
7723432	GTGCCTTGAATTAAAGAATC	ACT
1546221	CTTATGTTGGAGAGGTC	ACG
4479801	TAGAAAGTAGTTGTGATCTTG	ACG
4395595	AAAAATGGTATACTGGACTTTA	ACG
4395596	CTGTGGCTTGTTGTTCT	ACT
4699932	CACTGGATTGTCCTCTTA	ACG
2936201	CCTAATGTCTAATGATGGTC	ACT
7356672	CACAGTGAGATGCCACA	ACG
2936200	CTTGGATAACTGTATGCCT	ACT
1909296	TTACATGAGTGCACCAC	CGT
7703131	GGTAATAAACTTTTCCGAG	ACT

dbSNP rs#	Extend Primer	Term Mix
7445308	GTTGGGACTATCTACTTTTTT	CGT
3087748	CATAGGAATCTGCTGCCTCAC	ACT
4321723	ACTTGGAACCCTGGTATTTT	ACG
2968016	GGGGCAAAAAGACAATAAA	ACG
5868151	GAATAGACTACATCCATCAAAATA	ACG
1874858	GAAACTTCCTTATCCAAGGCC	ACT
1874857	GCAATTAAAAGTGGTACAATAA	ACT
7712922	CAATAAAAGGAAAGAGACTTC	ACG
4631140	AGAGGGTCTCCTGTAGG	ACG
4469166	GAGGAATGGAACTATTTTTAAAC	ACT
1078369	CTCTGTTTTCCCTGTAAAT	ACT
1078368	GCAACAGGTGAAGCTGA	ACG
2968006	GACTGAACCTTTAGGAGA	ACT
2968005	TTCTCCTAAAGGTTCAGT	CGT
2936190	CCAACACAACCTCTTAA	ACG
2409613	CCAAGTGACTAAAGCAGAT	ACT
4415048	AAAACCTAATGCTCAGTTAAA	ACT
2968004	TCTGGCATAAAGTAAACTAATC	ACT
2968003	GGGGACTAGTCAACAAA	ACG
2968002	TCTGATGAGGTCTTCTAC	ACT
2936191	GGCTGAAGTAGTGATAGG	CGT
1498610	GAAGCACAGAAGGCAAC	ACT
6874662	CAGAGCGAGACTCCATC	CGT
3060393	GATTTGCTCCTAAGTTTTTT	ACT
7729722	GTGTCTATTCAGATCCTTTG	ACG
7733884	TTGAGGAAAGTGTGTCT	ACT
7714489	GGCTGGAGTGCAGTGGC	ACT
7735570	TCCACATTATGCAACTACA	ACT
2936193	AAAAGTCAGTGTAAGAGTTC	CGT
2291851	TGATGGCATTGAAGCAG	ACT
2291852	GTTTTTATTGTGCTTTTCAATCT	ACT
1498602	GAATTTGAGCAGCTAGTT	ACT
1995166	TGTGCAAGCATTAGTGTAT	ACT
1498603	GAGCAGAATCAAAAGCC	CGT
1498604	GCTCTTCATTTTCTTACACAG	CGT
1498605	AGGCACCTTTATTCTCAT	ACG
1948651	GAATAAAGGTGCCTAATGC	ACG
4699934	GTAATAGTTGCCTCGTC	CGT
4700319	TTACTAATTGTCTCACAACATTA	ACG
2279737	TCGCCGGCATGGGAATC	ACT
7720361	AGTCCCAGTCCAGGAGC	ACG
7706419	TTCATTCACTTCAAGTGC	CGT
1006431	GTGAATTAAAGTTTCAGATTGAA	ACT
1353747	GGAGAGGAGCCACAGAA	CGT
1498606	CAAGTGATTTCCCAGTAG	ACG
1353748	ACCAAGTTCCCAAAGCT	ACT
1553113	GGAGTTTAGCCAATAGTTTTTGC	ACT
2968012	GACAGTATCAATTCCTTCC	ACT
2968011	TGACTTCAACTTTACTTTCTT	CGT
1498608	CCCTAAAAACTGTTCCAGGTA	CGT
2936189	CCAGAACTGTGAGAAATAAA	CGT

dbSNP rs#	Extend Primer	Term Mix
1498609	GTTTTCTATTTGAAATTGAGGTAT	ACG
2968019	CCAGTATTACTGTTTGAATCTC	ACT
6891238	CTGCAGACATTTCCTAC	ACT
2968010	TTCACTCTGATGTTTTCTATTT	CGT
2968009	CATAACCTCTGGTTTCC	ACG
2936203	GGAGGATATCCATGCCCC	ACT
1498601	AGCTCTGCCTCCCCGATTT	ACG
1498600	AGGTGCCAAGCTAGTCA	ACT
1498599	GGCTACATCATGTTTTGG	ACG
2936202	CCCTGGTAACTGTAGTG	ACT
7730070	TAGGCATGCTCAAACTC	ACT
6450501	GCCTCCTAAAAAAGCAC	ACT
6450502	GTTTAATGACATTTTAGAGAGG	CGT
6889456	AACCTCTCTAAAATGTCATTAA	ACT
6894618	GGACAAAGGAAATGTGATATATA	ACG
7706044	GGCTCATTTCCTGGGTC	ACT
7707541	GAAAAACTGACAACATATGAG	CGT
7712076	CCCAGAGGATATAGATTTCAT	ACG
6892860	GGATATTTCAGTGGTGCT	ACG
6867053	CACCATTAACCTGGTCTA	ACT
7737269	ACTCTTGATCAATAGTTTGG	ACG
6864156	GTAGTTGACAAATCTTACCT	ACT
950447	TTGTTAGTGTATTAGCCATTG	ACT
2936196	GTGCAAAGATCAATCACA	ACT
7719347	GGTCATGCAGGAAATAG	ACT
1391649	TCTGGTCTATTTCCTGC	CGT
1391650	CCCACAGGTTTTTCACA	ACT
1391651	GGGCCCCCGTTTTGCCCTG	ACG
1353749	CAAAAAGCCAAGGATATAAAATA	ACG
10882149	GTATGATCTGATTTCCATAAATAG	ACT
5868153	GAAATGAATAAATGGCAAAAAA	ACT
1363882	GCAATGAGTAGACTAAAAAAA	ACT
2409626	AGCAATCATCCCACCTC	ACT
2968018	CAGACACCCACTGACCA	ACG
954740	ACGAAGGTCCCGTCTGA	ACT
986067	CAGGGATTTAAAATGAAACC	ACT
6869400	AATGGTGGGTCATTAGTT	ACT
5010782	GCACCTACAGAGGAAAT	CGT

Genetic Analysis

[0248] Allelotyping results are shown for female cases and controls in Table 23. The allele frequency for the A2 allele is noted in the fifth and sixth columns for control pools and case pools, respectively, where "AF" is allele frequency. Some SNPs do not have an allele frequency disclosed because of failed assays.

TABLE 23

dbSNP rs#	Position in SEQ ID NO:4	Chromosome Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
6886495	249	58309549	G/C	G=	G=0.87			
				C=	C=0.13			
6450498	543	58309843	A/T	A=0.81	A=0.85	0.1780	1.29	A
				T=0.19	T=0.15			
1472456	973	58310273	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
4700315	1076	58310376	A/G	A=	A=0.95			
				G=	G=0.05			
4700316	1276	58310576	G/C	G=0.87	G=0.84	0.1520	0.75	C
				C=0.13	C=0.16			
7714708	1599	58310899	A/G	A=0.35	A=0.46	0.0013	1.60	A
				G=0.65	G=0.54			
7710479	2755	58312055	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
2968013	2911	58312211	G/C	G=0.84	G=0.80	0.1256	0.79	C
				C=0.16	C=0.20			
2968014	4466	58313766	A/G	A=0.22	A=0.25	0.2834	1.16	A
				G=0.78	G=0.75			
2968015	5754	58315054	T/C	T=0.88	T=0.86	0.2038	0.80	C
				C=0.12	C=0.14			
1391648	5762	58315062	A/G	A=0.25	A=0.24	0.8566	0.97	G
				G=0.75	G=0.76			
2055297	5967	58315267	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
2055296	5972	58315272	A/G	A=0.94	A=0.94	0.8857	1.04	A
				G=0.06	G=0.06			
3989138	6390	58315690	-/AA	-=0.53	-=0.50	0.3703	0.90	A
				AA=0.47	AA=0.50			
4700317	6984	58316284	C/T	C=0.00	C=0.00			
				T=1.00	T=1.00			
2036220	7234	58316534	G/A	G=0.44	G=0.44	0.9272	1.01	G
				A=0.56	A=0.56			
7727206	8196	58317496	G/T	G=0.27	G=0.25	0.3954	0.89	T
				T=0.73	T=0.75			
7723432	8369	58317669	A/G	A=0.82	A=0.82	0.7671	1.05	A
				G=0.18	G=0.18			
1546221	9565	58318865	C/T	C=0.83	C=0.84	0.7282	1.06	C
				T=0.17	T=0.16			
4479801	11084	58320384	C/T	C=0.86	C=0.87	0.7440	1.07	C
				T=0.14	T=0.13			
4395595	11153	58320453	C/T	C=0.93	C=0.93	0.7241	1.09	C
				T=0.07	T=0.07			
4395596	11187	58320487	T/C	T=0.01	T=0.02			
				C=0.99	C=0.98			
4699932	11290	58320590	G/A	G=0.13	G=0.10	0.1440	0.76	A
				A=0.87	A=0.90			

dbSNP rs#	Position in SEQ ID NO:4	Chromosome Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
2936201	11386	58320686	A/G	A=0.17	A=0.19	0.3609	1.15	A
				G=0.83	G=0.81			
7356672	11441	58320741	C/T	C=0.88	C=0.89	0.4757	1.17	C
				T=0.12	T=0.11			
2936200	12373	58321673	A/G	A=1.00	A=1.00			
				G=0.00	G=0.00			
1909296	12602	58321902	C/A	C=0.98	C=0.99			
				A=0.02	A=0.01			
7703131	13763	58323063	C/G	C=	C=			
				G=	G=			
7445308	18697	58327997	A/T	A=0.00	A=0.00			
				T=1.00	T=1.00			
3087748	18854	58328154	T/C	T=	T=0.21			
				C=	C=0.79			
4321723	19107	58328407	C/T	C=0.19	C=0.24	0.1033	1.30	C
				T=0.81	T=0.76			
2968016	19310	58328610	C/T	C=0.45	C=0.44	0.7959	0.96	T
				T=0.55	T=0.56			
5868151	20074	58329374	C/-	C=0.44	C=0.43	0.6659	0.95	-
				-=0.56	-=0.57			
1874858	20145	58329445	T/C	T=0.12	T=0.17	0.1558	1.43	T
				C=0.88	C=0.83			
1874857	20281	58329581	A/C	A=0.96	A=0.96	0.5485	0.83	A
				C=0.04	C=0.04			
7712922	23117	58332417	C/T	C=0.05	C=0.00			
				T=0.95	T=1.00			
4631140	23585	58332885	G/A	G=0.10	G=0.09	0.4903	0.87	A
				A=0.90	A=0.91			
4469166	23906	58333206	T/C	T=0.97	T=0.98	0.6510	1.30	T
				C=0.03	C=0.02			
1078369	24046	58333346	C/G	C=0.88	C=0.88	0.8279	0.96	C
				G=0.12	G=0.12			
1078368	24450	58333750	G/A	G=0.50	G=0.47	0.3033	0.89	A
				A=0.50	A=0.53			
2968006	24619	58333919	A/G	A=0.34	A=0.35	0.5216	1.08	A
				G=0.66	G=0.65			
2968005	24637	58333937	G/T	G=0.37	G=0.36	0.7332	0.96	T
				T=0.63	T=0.64			
2936190	24894	58334194	C/T	C=0.22	C=0.22	0.9071	1.02	C
				T=0.78	T=0.78			
2409613	25030	58334330	G/C	G=1.00	G=1.00			
				C=0.00	C=0.00			
4415048	25732	58335032	G/C	G=0.94	G=0.96	0.2666	1.36	G
				C=0.06	C=0.04			
2968004	27106	58336406	A/C	A=0.92	A=0.88	0.1210	0.70	C
				C=0.08	C=0.12			
2968003	27395	58336695	G/A	G=0.61	G=0.66	0.2364	1.23	G
				A=0.39	A=0.34			
2968002	28971	58338271	A/G	A=0.81	A=0.81	0.8196	1.04	A

dbSNP rs#	Position in SEQ ID NO:4	Chromosome Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
				G=0.19	G=0.19			
2936191	29755	58339055	T/A	T=1.00	T=1.00			
				A=0.00	A=0.00			
1498610	30988	58340288	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
6874662	31827	58341127	C/A	C=0.00	C=0.00			
				A=1.00	A=1.00			
3060393	31843	58341143	-/CA	-=1.00	-=1.00			
				CA=0.00	CA=0.00			
7729722	32773	58342073	G/A	G=0.01	G=0.01			
				A=0.99	A=0.99			
7733884	32787	58342087	T/C	T=0.96	T=0.97	0.5282	1.26	T
				C=0.04	C=0.03			
7714489	33099	58342399	T/C	T=0.15	T=0.14	0.7668	0.95	C
				C=0.85	C=0.86			
7735570	36854	58346154	T/C	T=0.08	T=0.09	0.4593	1.18	T
				C=0.92	C=0.91			
2936193	38026	58347326	G/T	G=0.25	G=0.28	0.3638	1.14	G
				T=0.75	T=0.72			
2291851	38397	58347697	T/C	T=0.05	T=0.06	0.2114	1.40	T
				C=0.95	C=0.94			
2291852	38680	58347980	A/G	A=0.98	A=0.98			
				G=0.02	G=0.02			
1498602	39626	58348926	T/C	T=0.67	T=0.61	**0.0496**	0.77	C
				C=0.33	C=0.39			
1995166	39682	58348982	T/C	T=0.54	T=0.55	0.7840	1.03	T
				C=0.46	C=0.45			
1498603	39710	58349010	G/T	G=0.03	G=0.02			
				T=0.97	T=0.98			
1498604	39745	58349045	C/A	C=0.16	C=0.13	0.2329	0.79	A
				A=0.84	A=0.87			
1498605	39901	58349201	G/A	G=0.88	G=0.89	0.3713	1.18	G
				A=0.12	A=0.11			
1948651	39925	58349225	C/T	C=0.58	C=0.56	0.5199	0.93	T
				T=0.42	T=0.44			
4699934	40356	58349656	G/T	G=0.13	G=0.05	**0.0043**	0.35	T
				T=0.87	T=0.95			
4700319	40393	58349693	C/T	C=	C=0.98			
				T=	T=0.02			
2279737	41230	58350530	A/G	A=0.82	A=0.80	0.3520	0.87	G
				G=0.18	G=0.20			
7720361	41733	58351033	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
7706419	41877	58351177	G/T	G=0.89	G=0.92	0.1509	1.38	G
				T=0.11	T=0.08			
1006431	43555	58352855	T/G	T=0.22	T=0.30	**0.0077**	1.49	T
				G=0.78	G=0.70			
1353747	44066	58353366	G/T	G=0.23	G=0.15	**0.0025**	0.61	T
				T=0.77	T=0.85			

dbSNP rs#	Position in SEQ ID NO:4	Chromosome Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
1498606	44134	58353434	C/T	C=0.85	C=0.87	0.3475	1.17	C
				T=0.15	T=0.13			
1353748	44181	58353481	T/G	T=0.65	T=0.66	0.8645	1.02	T
				G=0.35	G=0.34			
1553113	45022	58354322	A/C	A=0.95	A=0.97	0.1730	1.54	A
				C=0.05	C=0.03			
2968012	46856	58356156	T/G	T=0.66	T=0.66	0.9069	1.01	T
				G=0.34	G=0.34			
2968011	48231	58357531	C/A	C=0.67	C=			
				A=0.33	A=			
1498608	49652	58358952	A/T	A=0.19	A=0.13	0.0118	0.64	T
				T=0.81	T=0.87			
2936189	50393	58359693	T/A	T=0.63	T=0.65	0.6021	1.08	T
				A=0.37	A=0.35			
1498609	51103	58360403	C/T	C=0.34	C=0.40	0.0314	1.30	C
				T=0.66	T=0.60			
2968019	51733	58361033	T/C	T=0.62	T=0.64	0.5417	1.08	T
				C=0.38	C=0.36			
6891238	54733	58364033	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
2968010	57173	58366473	A/T	A=0.90	A=0.95	0.0012	2.28	A
				T=0.10	T=0.05			
2968009	58192	58367492	C/T	C=0.65	C=0.70	0.0959	1.24	C
				T=0.35	T=0.30			
2936203	58506	58367806	G/C	G=0.17	G=0.14	0.1730	0.80	C
				C=0.83	C=0.86			
1498601	59572	58368872	G/A	G=0.81	G=0.78	0.3899	0.83	A
				A=0.19	A=0.22			
1498600	59738	58369038	A/G	A=0.48	A=0.46	0.5991	0.94	G
				G=0.52	G=0.54			
1498599	61617	58370917	G/A	G=0.69	G=0.69	0.9739	1.00	G
				A=0.31	A=0.31			
2936202	63980	58373280	C/G	C=0.20	C=0.14	0.0286	0.68	G
				G=0.80	G=0.86			
7730070	64161	58373461	C/G	C=0.74	C=0.73	0.5744	0.93	G
				G=0.26	G=0.27			
6450501	66871	58376171	A/G	A=0.00	A=0.09			
				G=1.00	G=0.91			
6450502	67063	58376363	A/T	A=1.00	A=1.00			
				T=0.00	T=0.00			
6889456	67084	58376384	A/G	A=	A=1.00			
				G=	G=0.00			
6894618	67477	58376777	C/T	C=0.69	C=0.73	0.1542	1.21	C
				T=0.31	T=0.27			
7706044	69282	58378582	T/C	T=	T=1.00			
				C=	C=0.00			
7707541	70363	58379663	A/T	A=0.63	A=0.61	0.5874	0.93	T
				T=0.37	T=0.39			
7712076	70647	58379947	C/T	C=0.00	C=0.00			

dbSNP rs#	Position in SEQ ID NO:4	Chromosome Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
				T=1.00	T=1.00			
6892860	71834	58381134	C/T	C=0.81	C=0.83	0.4091	1.14	C
				T=0.19	T=0.17			
6867053	72130	58381430	C/G	C=0.46	C=0.44	0.4563	0.91	G
				G=0.54	G=0.56			
7737269	73495	58382795	C/T	C=0.00	C=0.00			
				T=1.00	T=1.00			
6864156	74542	58383842	T/C	T=0.99	T=0.99			
				C=0.01	C=0.01			
950447	75280	58384580	T/C	T=	T=1.00			
				C=	C=0.00			
2936196	80740	58390040	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
7719347	82579	58391879	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
1391649	82591	58391891	C/A	C=0.42	C=0.35	0.0162	0.74	A
				A=0.58	A=0.65			
1391650	82976	58392276	T/C	T=0.75	T=0.75	0.9568	0.99	T
				C=0.25	C=0.25			
1391651	83040	58392340	G/A	G=0.58	G=0.59	0.8831	1.02	G
				A=0.42	A=0.41			
1353749	85894	58395194	G/A	G=0.43	G=0.39	0.1596	0.85	A
				A=0.57	A=0.61			
1068214 9	86020	58395320	-/CCT	-=0.37	-=0.39	0.5382	1.08	-
				CCT=0.63	CCT=0.61			
5868153	86947	58396247	A/-	A=0.72	A=0.71	0.7470	0.96	-
				-=0.28	-=0.29			
1363882	88922	58398222	C/G	C=0.45	C=0.43	0.5228	0.93	G
				G=0.55	G=0.57			
2409628	89662	58398962	T/C	T=0.84	T=0.85	0.8581	1.03	T
				C=0.16	C=0.15			
2968018	92367	58401667	G/A	G=0.75	G=0.75	0.8411	0.97	G
				A=0.25	A=0.25			
954740	93154	58402454	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
986067	94979	58404279	T/C	T=0.04	T=0.06	0.2475	1.43	T
				C=0.96	C=0.94			
6869400	97598	58406898	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
5010782	98532	58407832	T/A	T=0.68	T=0.69	0.8644	1.02	T
				A=0.32	A=0.31			

[0249] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 4. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in

the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 4 can be determined by consulting Table 23. For example, the left-most X on the left graph is at position 58309549. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0250] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0251] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is place at the 3' end of each gene to show the direction of transcription.

Example 9

GPX3 Proximal SNPs

[0252] It has been discovered that a polymorphic variation (rs869975) in a gene encoding *GPX3* is associated with the occurrence of low BMD (see Examples 1 and 2). Two hundred thirty-three additional allelic variants proximal to rs869975 were identified and subsequently allelotyped in low BMD case and high BMD control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 24. The chromosome position provided in column four of Table 24 is based on Genome "Build 34" of NCBI's GenBank.

TABLE 24

dbSNP	Position in SEQ ID NO:5	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_Iupac
1478398	231	5	150385031	a/g	t	Y
1478397	330	5	150385130	t/c	a	R
1160114	582	5	150385382	g/c	g	S
1160113	589	5	150385389	a/g	c	Y

dbSNP	Position in SEQ ID NO:5	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
1382323	1060	5	150385860	a/g	c	Y
1160112	1066	5	150385866	g/a	t	Y
7709870	1311	5	150386111	g/a	a	R
7710643	1556	5	150386356	g/t	g	K
7730467	1655	5	150386455	t/c	t	Y
6579829	1692	5	150386492	a/c	a	M
6579830	1802	5	150386602	g/a	g	R
6579831	2061	5	150386861	t/a	a	W
6896232	2112	5	150386912	t/a	t	W
1351131	2153	5	150386953	t/c	g	R
1038074	2667	5	150387467	c/t	g	R
1478396	3115	5	150387915	t/c	a	R
6880512	3186	5	150387986	g/a	a	R
4958858	5621	5	150390421	t/c	t	Y
4958431	5735	5	150390535	t/g	g	K
4958432	5829	5	150390629	g/c	g	S
6898463	6658	5	150391458	a/c	c	M
4958859	7901	5	150392701	g/c	c	S
4130064	11447	5	150396247	g/a	g	R
4130065	11466	5	150396266	a/g	a	R
4133119	11984	5	150396784	t/c	g	R
4958860	15803	5	150400603	t/g	t	K
4958861	16257	5	150401057	t/g	t	K
4437356	17604	5	150402404	c/a	c	M
4958868	19762	5	150404562	c/t	t	Y
1478400	22367	5	150407167	a/g	g	R
6889375	22709	5	150407509	a/g	g	R
1600159	23631	5	150408431	g/c	c	S
6875892	23686	5	150408486	t/a	t	W
4608909	25599	5	150410399	t/c	t	Y
2345000	26973	5	150411773	a/c	c	M
4516840	28457	5	150413257	g/t	t	K
2054440	28669	5	150413469	a/g	a	R
707141	29908	5	150414708	c/t	a	R
707142	30105	5	150414905	a/g	t	Y
841236	30711	5	150415511	a/g	a	R
707143	30851	5	150415651	g/a	t	Y
707144	31203	5	150416003	t/c	g	R
6869405	31446	5	150416246	a/c	c	M
707145	31638	5	150416438	c/t	g	R
707146	33064	5	150417864	c/t	g	R
707148	33958	5	150418758	c/a	t	K
707150	35182	5	150419982	a/t	t	W
5872184	38332	5	150423132	a/ac	a	N
3763015	40875	5	150425675	g/a	c	Y
2042235	41624	5	150426424	t/c	t	Y
3763013	41671	5	150426471	a/g	c	Y
2042236	41825	5	150426625	g/a	g	R
1946234	42920	5	150427720	c/a	a	M
1946235	42935	5	150427735	t/c	t	Y
1946236	43001	5	150427801	t/a	a	W

dbSNP	Position in SEQ ID NO:5	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_Iupac
8177402	43012	5	150427812	t/c	c	Y
8177403	43203	5	150428003	c/t	c	Y
8177404	43294	5	150428094	t/c	t	Y
8177405	43295	5	150428095	t/c	c	Y
8177406	43344	5	150428144	c/t	t	Y
8177407	43509	5	150428309	t/c	c	Y
8177408	43549	5	150428349	g/c	c	S
8177409	43560	5	150428360	t/a	a	W
6888961	43578	5	150428378	a/t	t	W
8177410	43640	5	150428440	a/g	g	R
8177411	43792	5	150428592	g/c	g	S
8177412	43797	5	150428597	c/t	t	Y
8177413	43964	5	150428764	c/g	g	S
870407	44297	5	150429097	c/t	a	R
870406	44311	5	150429111	c/t	g	R
6873202	44588	5	150429388	a/g	a	R
8177414	44775	5	150429575	c/t	c	Y
8177415	44921	5	150429721	c/t	c	Y
3805435	45006	5	150429806	g/a	t	Y
8177416	45098	5	150429898	t/c	c	Y
3792799	45185	5	150429985	c/g	c	S
3792798	45475	5	150430275	t/c	g	R
3828599	45506	5	150430306	t/c	a	R
8177417	45543	5	150430343	g/c	g	S
3792797	45601	5	150430401	t/g	a	M
8177418	45652	5	150430452	t/c	c	Y
8177419	45756	5	150430556	ag/g	g	N
8177420	45826	5	150430626	t/c	t	Y
8177421	45974	5	150430774	c/g	g	S
4958872	46044	5	150430844	t/c	c	Y
3792796	46200	5	150431000	c/g	g	S
8177422	46218	5	150431018	a/g	a	R
8177423	46221	5	150431021	c/t	c	Y
4958434	46280	5	150431080	c/t	a	R
8177424	46330	5	150431138	-/gagtcctgg	gagtcctgg	N
8177425	46583	5	150431383	t/c	c	Y
8177426	46650	5	150431450	a/g	a	R
8177427	46721	5	150431521	a/g	a	R
8177429	46808	5	150431608	g/c	g	S
6889737	47242	5	150432042	c/a	c	M
3792795	47512	5	150432312	g/a	c	Y
8177430	47600	5	150432400	t/c	c	Y
8177431	47706	5	150432506	a/g	g	R
4958873	47806	5	150432606	a/g	a	R
8177432	47978	5	150432778	t/g	t	K
8177433	48021	5	150432821	t/c	c	Y
8177434	48025	5	150432825	g/a	a	R
8177435	48093	5	150432893	t/g	g	K
3763011	48413	5	150433213	t/c	g	R
8177436	48933	5	150433733	c/t	t	Y
8177437	49097	5	150433897	c/g	g	S

dbSNP	Position in SEQ ID NO:5	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
4958874	49105	5	150433905	t/c	t	Y
8177439	49570	5	150434370	g/a	g	R
8177440	49591	5	150434391	c/t	c	Y
8177441	49704	5	150434504	g/c	g	S
8177442	49705	5	150434505	a/t	a	W
8177443	49798	5	150434598	c/t	c	Y
869975	50082	5	150434882	a/g	g	R
869976	50147	5	150434947	g/a	a	R
8177444	50356	5	150435156	a/t	a	W
8177445	50725	5	150435525	t/c	t	Y
7721469	50968	5	150435768	c/a	a	M
8177446	51029	5	150435829	a/c	a	M
7704191	51086	5	150435886	t/c	t	Y
8177447	51166	5	150435966	t/c	t	Y
11548	51493	5	150436293	c/t	c	Y
2230303	51539	5	150436339	g/t	t	K
7722386	51562	5	150436362	g/a	a	R
8177448	51645	5	150436445	g/a	g	R
8177449	51649	5	150436449	t/c	c	Y
2070593	51650	5	150436450	t/c	g	R
8177450	51656	5	150436456	g/a	a	R
8177451	51657	5	150436457	t/c	c	Y
8177452	52009	5	150436809	g/a	a	R
8177453	52143	5	150436943	a/c	c	M
8177454	52349	5	150437149	g/c	g	S
3763010	52421	5	150437221	c/t	c	Y
8177455	52532	5	150437332	a/g	g	R
8177456	52682	5	150437482	a/g	g	R
736775	53058	5	150437858	t/c	t	Y
2277940	53187	5	150437987	t/c	t	Y
8177458	53377	5	150438177	g/a	g	R
8177834	53699	5	150438499	g/a	c	Y
3924	53845	5	150438645	a/g	g	R
2233312	53920	5	150438720	a/g	c	Y
2233311	53929	5	150438729	t/g	c	M
2233310	55473	5	150440273	t/c	g	R
2233309	55690	5	150440490	a/g	c	Y
4958875	55850	5	150440650	a/g	g	R
2233308	56761	5	150441561	c/t	g	R
2233307	56840	5	150441640	c/g	c	S
2233306	57000	5	150441800	c/t	g	R
2233305	57116	5	150441916	t/g	a	M
2233304	58419	5	150443219	t/g	c	M
2233303	58420	5	150443220	c/t	g	R
2233302	58808	5	150443608	g/c	c	S
2287719	58906	5	150443706	a/g	g	R
2287720	59048	5	150443848	c/t	c	Y
7727034	59187	5	150443987	c/g	c	S
7727250	59361	5	150444161	c/t	c	Y
7709800	61218	5	150446018	g/a	g	R
3840312	61700	5	150446500	ag/a	a	N

dbSNP	Position in SEQ ID NO:5	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
2287721	62290	5	150447090	g/a	g	R
6875293	62596	5	150447396	t/c	c	Y
3805434	64049	5	150448849	g/c	g	S
2080982	66077	5	150450877	g/t	t	K
2080983	66079	5	150450879	g/t	g	K
2287722	66086	5	150450886	t/c	c	Y
2233301	66115	5	150450915	t/g	c	M
2233300	66150	5	150450950	c/g	c	S
4958876	66475	5	150451275	c/a	a	M
2233299	69177	5	150453977	a/g	g	R
2233298	69210	5	150454010	a/g	c	Y
2287723	69312	5	150454112	t/g	t	K
2161359	70244	5	150455044	a/g	g	R
7734456	70882	5	150455682	g/c	g	S
4292439	71905	5	150456705	t/c	c	Y
4958878	72294	5	150457094	a/t	a	W
6862024	72581	5	150457381	a/g	g	R
3834819	72786	5	150457589	-/ca	ca	N
2233297	72950	5	150457750	g/a	t	Y
2233296	73106	5	150457906	g/a	c	Y
2233295	73162	5	150457962	t/c	g	R
2233294	73273	5	150458073	g/t	a	M
7713028	74131	5	150458931	g/a	g	R
7713223	74406	5	150459206	t/c	c	Y
7713567	74665	5	150459465	c/t	c	Y
888989	74740	5	150459540	t/c	c	Y
2233293	75382	5	150460182	a/g	c	Y
3749657	75400	5	150460200	g/t	c	M
2233292	75460	5	150460260	g/a	c	Y
2112635	75863	5	150460663	c/t	c	Y
871269	76098	5	150460898	t/c	c	Y
3792794	78432	5	150463232	a/g	c	Y
6579837	78604	5	150463404	g/t	g	K
3805433	79190	5	150463990	c/g	c	S
5872186	79870	5	150464670	-/a	a	N
2233291	79928	5	150464728	g/c	c	S
2233290	80213	5	150465013	g/c	g	S
2233289	80227	5	150465027	c/t	g	R
4958435	81994	5	150466794	t/g	g	K
4958880	82187	5	150466987	c/a	c	M
1422673	82698	5	150467498	t/c	c	Y
2042234	82841	5	150467641	a/g	a	R
3805432	83214	5	150468014	a/g	c	Y
3805431	83249	5	150468049	t/c	g	R
2233288	83485	5	150468285	t/c	g	R
2233287	83807	5	150468607	c/t	g	R
3815720	83907	5	150468707	g/a	g	R
3792792	84216	5	150469016	a/g	t	Y
3792791	84656	5	150469456	a/g	c	Y
2303018	85448	5	150470248	g/a	g	R
3792790	85881	5	150470681	g/t	a	M

dbSNP	Position in SEQ ID NO:5	Chromosome	Chromosome Position	Alleles (A1/A2)	genome_letter	deduced_iupac
4958436	86539	5	150471339	t/c	t	Y
2233286	86796	5	150471596	t/c	g	R
2233285	87057	5	150471857	g/a	c	Y
7732451	87922	5	150472722	t/c	a	R
2233284	88098	5	150472898	t/c	g	R
1422674	89319	5	150474119	g/t	t	K
3792789	89678	5	150474478	c/t	g	R
4562032	90026	5	150474826	c/a	c	M
6865077	90033	5	150474833	g/a	g	R
1559126	90114	5	150474914	c/g	c	S
3792788	90326	5	150475126	t/c	g	R
1559127	90463	5	150475263	t/c	t	Y
3792786	90548	5	150475348	a/g	t	Y
6880110	90800	5	150475600	a/g	a	R
6861227	90838	5	150475638	g/t	t	K
3805430	91400	5	150476200	c/g	c	S
1862364	92086	5	150476886	a/g	a	R
4958881	93946	5	150478746	t/c	t	Y
3792785	95360	5	150480160	a/g	t	Y
6869605	96576	5	150481376	a/c	a	M
6870205	96721	5	150481521	a/g	g	R
4246047	98316	5	150483116	t/a	t	W
4958882	98497	5	150483297	c/g	c	S
3792784	99382	5	150484182	c/t	a	R
3792783	99442	5	150484242	t/c	a	R
5872188	99764	5	150484565	-/ag	ag	N

Assay for Verifying and Allelotyping SNPs

[0253] The methods used to verify and allelotype proximal SNPs of Table 24 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 25 and Table 26, respectively.

TABLE 25

DbSNP rs#	Forward PCR primer	Reverse PCR primer
1478398	TTCCCTCCCTTCCTTTCTTC	AAGATTACCCCACTGCACTC
1478397	CACATACTTGTCTGAGGATC	AGCACTCAGAATACTACCTG
1160114	AGCTGGATTCAGAGCCTTTG	TCTGCTATCACCTTGTTCCC
1160113	TCTGCTATCACCTTGTTCCC	AGCTGGATTCAGAGCCTTTG
1382323	AGAAGGACAGTTGCAACAGG	GTTCCTCATCCAGTCCTTAC
1160112	GTTCCTCATCCAGTCCTTAC	AGAAGGACAGTTGCAACAGG
7709870	TTATGACTCATAACTTTTTG	CTTTCTTGAAATGCTCAGGG
7710643	ATAAAGTCTGGGCTCAGATG	ATAGACCAGGGAACTGAGAC
7730467	TGTGGCATATCTTCTATTGC	AATGTGGCTGTTATGGTAGG
6579829	GGAGGCTTTTTCACATTCAC	AATGTGGCTGTTATGGTAGG
6579830	TGAGTATGGATTCTCAGGTG	ACCATAAGAGAGAAAAGCCC
6579831	GTGTGTGATGAATATGGGAG	TTTCTCTCCTGTGTGTATTC
6896232	GTCACACCTCTTTGTACATC	CACTGAAAGCTCTCTCACAC

DbSNP rs#	Forward PCR primer	Reverse PCR primer
1351131	AAGTGTGAGAGAGCTTTCAG	GTATAAGGTTTCTCTCTAGTG
1038074	TCATATCTTGTGGGTTGTCC	TTCACTGTGGACCACAAAGG
1478396	CATGGCCTCTTACAAGACTC	GGGTAATTGTACCCACCAAG
6880512	GAGGTATTGGTGATTAGGGC	TAAAAAGGAATTCTTGGTGG
4958858	GCAAGTGAGATCTTGGTAGG	CAGACTGGCAGGAGTACATT
4958431	CAGAGGTGTCACTTCAGGAA	CCATCTTGCTTCTTTCCCTG
4958432	TGCCTTAATTCATGCAACCC	TTATATCCAGGGAAAGAAGC
6898463	CAAATCTACTTTTGAGTACC	GACTCATCAAATGGAGAATG
4958859	GTGGTATAACTCCTCAGAAG	ATGATGAAGTTGTCTTCAGG
4130064	AATTCCTCACATTAACCCAG	CCACAGCAAAGACAAAAGGG
4130065	CCACAGCAAAGACAAAAGGG	TCCTCACATTAACCCAGGAG
4133119	AGTGAGGTATAATGCCAGCC	AAAATCTCCCTGTGTCTGCC
4958860	TATGCGGTGGAGAAGAAAGG	TCAACCCGGACAACAAAACC
4958861	ACTGGGATTTTCTGGTGAAG	ATGGGTGAGTCTCCCTTTAC
4437356	AGGTTTCTGGGTTTTCTCTC	ATGGTAGTGTGTTTGTTTGC
4958868	TTGTTGGCCTTGTTCATGTC	CCTGTAATCCCTGCACTTTG
1478400	TGGACTCCTGTGGGATAATG	CAAGACTTCCAAGTGCATCC
6889375	TACCTGTCCACAGAAATAGC	CCTGAGACCCTATGACTTTA
1600159	GGTTCATGTGATTTGGAAGC	TGACTCTCCTAGTTCTTCAG
6875892	GGGACTTCTCAGCTTCCAAA	AGGGTTCTCCAGAGAAACAG
4608909	CTGATAGAGGTTTATCTCAGC	TCTAGCCAAACTCCTATGTG
2345000	TAACAAGCCCCTAACTAGTC	TCAGGTGAAACCAACTGAAG
4516840	GTGCAGTGGATCCTTTTTCC	CTAGACTCTAGGTAGGACAC
2054440	CCCTTGAATGAGATGGAGAC	TGGGAAAGGAAGGGAATGTC
707141	ACAGTGCTAAGCACTTTCCC	ACAGTAGACACACAGGATAA
707142	AGCCTGGCTTCTTCGTCTAC	ATTTCCACCCTGGGCACTTC
841236	ATGTGTGCATGAGAGGAGAG	CCAAAAAGGAGGAATGTGGG
707143	CTTTTAAAGCCAGATGGACC	CCACCCTGTTCTTACTGTAG
707144	ACTGCACCAGACGCTTTCAC	TTGGGCAAGGCGTGTTTCCT
6869405	TCTCTCATCATGGCTTGTGG	CTGGCCAAGCGATAACACTA
707145	TAAAGTCTGGGGAGGTCAGG	AGTGGCTGCTTTCGGAACAG
707146	GCAACAAGAGCGAAACTCTG	AAATGGGCAACATGGCAAAG
707148	ACCTTTCATATACAGCTGGG	CTGTGTTCCAGCATCATCTG
707150	ACTGATCTGGGCTAGGAATC	AAGGACAGCCAATTAACACG
5872184	TAAAATGTTGTTTACCAGGG	AAGGATGCCTATGTGACCAG
3763015	GCCAAATATTGTTTTCCTTG	ACATCGACCCTTTGTTGTAC
2042235	TCCTTCTCTGATCTCAGTTG	TTAGGAATGAAAAGCCACAG
3763013	AGAGACCTGAGATGCTACCC	ACAGGTGGACAACTGAGATC
2042236	AAAGGTGTTTCCAGCAGATA	AAAATGTAGAGAGATGCCCG
1946234	AACAAGTCATCTAGCCAGAG	CTGGCTTAGAAGCAGAAAAT
1946235	CCTGGCTTAGAAGCAGAAAA	GGCACTGGAGATCACTAATC
1946236	GCACTGGAGATCACTAATC	CAAACCACCTGGCTTAGAAG
8177402	TATCTGTGGCCAAACCACCT	CACAGGGAGCAAATTTATT
8177403	TTTCCTTGAGTCTTTGGGTC	GCCTGTAGAAAAAGGTTAGC
8177404	ATTAGGACTGTAGGGACAGA	GCTAACCTTTTTCTACAGGC
8177405	ATTAGGACTGTAGGGACAGA	TTTTCTACAGGCATGTCAGC
8177406	ATGTCAGCTGTGATCCTTAT	GAAAACCCCATTCTGGGTAG
8177407	TGATGGAAGTCTCCATTCTG	AATTTCCAGAGGACCATCGC
8177408	GGGCTAATAGCTCCCTAGAA	CGCCAGGTGTTTTTAAAACC
8177409	ATGCTTCCCAGAATGGAGAC	TCCATTACAGCCAGGGCAAG
6888961	TCCTCTTTTGGCTCCAAAGT	TTCCATCAGTTCTAGGGAGC
8177410	TCCTTTCGCACTTTGGAGCC	ATAAGCAAATCTGGGCAGAG
8177411	TGTGACCAATCCGCGGCCAA	TGAAATCCCAGCCGCCTAGC

DbSNP rs#	Forward PCR primer	Reverse PCR primer
8177412	TGAAATCCCAGCCGCCTAG	TTTCAAGCCCTCGGGTGTGAC
8177413	TGCAGGCGTCCTGCCTGCT	TCACTCACCTTCGACTTCTC
870407	GTGACATAGATGTAGCAAGG	AGAATGACTAAGGGAGGAAG
870406	CGGAGGTGACATAGATGTAG	AGAATGACTAAGGGAGGAAG
6873202	TAGAAATGGCCACTTGGATT	AGTGTTCTGCACTGTCACTC
8177414	GGGATGCTTGGAGAAGCTGA	AATGAGGACAGTGGGTCCTG
8177415	CCTCAGTCTATTTCCAGCAG	ATAAGCATGCTGGGTCATCC
3805435	GACAGTATGTACTGGGGTGG	TTTGAGTCTATCCCTACACC
8177416	GAAGACCCAGAGGATTTTAG	AGGGATAGACTCAAAGCGAG
3792799	GGACAGAATTTTGGAAACGG	CTAAAATCCTCTGGGTCTTC
3792798	ATTTTCCAGGTCAGGAATGG	CCAAAACTGAAGGTTGGGAC
3828599	AGTCAGTCCCAACCTTCAGT	CAGGGCCCAATTGTATCTTC
8177417	AGTTATTCATGGAGTCCACG	TTTTGGAAGGGTCAAAGAAG
3792797	GAGTCCATTTATCCTTCCCC	ACGTGGACTCCATGAATAAC
8177418	AAGTCCTGACTGGTTGTATG	CTCAGAGAGAATAGGATGGG
8177419	ATGCCCATCTCTGCTCCCAA	TCCTAACCCAGAGCTAAGAC
8177420	ATTCCATTCTCCACATGCCC	TAAGAGTTCAGATGTCTTAG
8177421	ATTGAAAGGGAGATGCTAGG	TTGTAGAGACAAGGAGCGTG
4958872	AGTCACCTAGAAACCAGCAG	ACAAAAGGACTGCAGAAGCC
3792796	CAATATGATGCTGTGAGAAG	ATCTTACAATGGAGGAGCTG
8177422	ATCTTACAATGGAGGAGCTG	TATGATGCTGTGAGAAGTGG
8177423	ATCTTACAATGGAGGAGCTG	TATGATGCTGTGAGAAGTGG
4958434	CTGCTGGTCTTGTGTCTTTC	AGACCACTTCTCACAGCATC
8177424	GGCTTGAGAGTTGGATGATG	GGAGAGGAGAAAGACACAAG
8177425	AGAGCAAAACAGGCCTGGAG	ACCCATGGGCCACCTTCTGA
8177426	ATTTCGTTCCAAGAGACTCC	ATATGTCCAGGGAGATAAGC
8177427	TTATCTCCCTGGACATATGC	GGCAAGGAGAACCCTATTTC
8177429	CAGGTTGTCCTAATAATGGG	ATGAAATAGGGTTCTCCTTG
6889737	CATGACAGAGGCAGCTCTTC	TGAAGGGAGTGATATATGCT
3792795	GGCCACAAAGGACCTTAAAG	TGACACAATTTGGCAGGTGG
8177430	AGTAGGATGTGGCCTATGAG	ATTGTGTCAATGATGCAATG
8177431	CATGATCCTATCAAGCCATC	GACCTGAGTTTCCTCATTTA
4958873	GCCAGTCCAAGACCACACAG	TGGGAGACCTGGGCTCCAT
8177432	CCCGGAACATTAACACATGC	TTACCAAATAGGGAAGCAGG
8177433	GGACAGGAAAGATGAAGCTG	ATGCAGGTACTGTTGCTTTT
8177434	GGACAGGAAAGATGAAGCTG	ATGCAGGTACTGTTGCTTTT
8177435	ATTGGGAGCCTCTGAATCAC	GGCCAGTGTGAAGGAAAAAC
3763011	GTGCTTTTTAAGCACTTAAT	CTCCTAATTCCTTGTTTAAG
8177436	CACTGTGTTCCGTGGTTTTG	CCTAGAACATGTCCAAAGGC
8177437	GAGATGGTAACTGAAGCTCC	TGCCAAGAGAAGCACCAAAG
4958874	TGCCAAGAGAAGCACCAAAG	GAGATGGTAACTGAAGCTCC
8177439	AGAGAAGCATCCCAGAAAAA	TCCATGCTCTGCCTGGCAAT
8177440	GCAATCACAGGCGAATTCCC	GCATCCCAGAAAAAGCACTC
8177441	TCCCCAGTTTCCTCAGTGTC	AACAGAGCAGAGTGCGTTTG
8177442	TCCCCAGTTTCCTCAGTGTC	AACAGAGCAGAGTGCGTTTG
8177443	TGCCAAAGATTATGGGAGGG	GAGCAAGAACTATCCATCCC
869975	CTTTCTCGGGCCTCAGTAGT	CTGTCTCATCCACACCACTC
869976	AGTGCATTCAGTTCTGGGCC	AGTGGTGTGGATGAGACAGG
8177444	AGGAGAGTAGTTCTTGGTGG	AGAAAGCTCCTCTCACATGG
8177445	TTCTCTCCATTGACATCCCC	TCAACAGGTATGTCCGACCA
7721469	ACTATTGTTCCAACTAGAGG	ATGTTCACTCTGGGTCCTTC
8177446	AGTTTGGGAGTTAGAAGACT	CAGAAGGACCCAGAGTGAAC
7704191	TTCCTGGGGAATAGCCTGGCC	CGCTGGCAGTCTTCTAACTC

DbSNP rs#	Forward PCR primer	Reverse PCR primer
8177447	TGGGAGGACAGGAGTTCTAG	GCCTCAAGCAAGGTTGACAC
11548	TGTTCAGGAAGAAATCCGTGT	GGGGCCTTGAGTGATAGGA
2230303	TGCCTGGCAGTACACAGAAC	ACTATCTACCCATCACAGAC
7722386	CCTGTAGGCATGTGTGTAAA	GCACAAATGGATGCATACAG
8177448	CAGATGGTACACATTCCCAG	GCCTACAGGTATGCGTGATT
8177449	CAGATGGTACACATTCCCAG	GCCTACAGGTATGCGTGATT
2070593	ACACATGCCTACAGGTATGC	CAGATGGTACACATTCCCAG
8177450	CAGGCACACAGATGGTACAC	GCCTACAGGTATGCGTGATT
8177451	CAGGCACACAGATGGTACAC	GCCTACAGGTATGCGTGATT
8177452	TGGCCCTTGCTGTCACATCT	TACATCCCCACCCCACAGTT
8177453	AGGGGTGGCATCCCTGCCCA	TGAGGGGCCAGCCCTTAGTG
8177454	TGCACCCACTGTAAAAAGT	AAGTGAGGAGGAGAAGGTGG
3763010	AGTGGGTGCAAAGGTAATTG	TGATCTCAGGGAGAATTGTG
8177455	ATGTAGCCCTATTAGGGGTG	AGCTAACTATCAGGTTGTTG
8177456	CCCGGGAGTTTGAGGCTATA	AGTGCAGTGGCTCTTCACAG
736775	TACTCTTGAGTAATGGTGAC	TGGCCTAGGCTCCTTCCACT
2277940	CGAATCATGGACATAAATCC	TCAGGGAAAGGAAGAAAAGG
8177458	ACAGTAGCCTTGCTGAAGCC	CAGCTTCAGGCTGGCGCCC
8177834	TCTTAGGATTGCTGCTCCTG	GAAAAACTCCTTCCCTGCTG
3924	TCCCCAGTCCTGTAAACAGC	GAGCCTCAGCTGGATGAGAG
2233312	ATTGTGTCATTTGGCTCCAC	CACACCGTGCAAGTGGCTTC
2233311	TTGTGTCATTTGGCTCCACC	CGTGCAAGTGGCTTCTAGTT
2233310	CACTGTTTTGGAAAGCTGGG	TTATCTGCCAGCATTGCCTG
2233309	ATTTAGTATTGTCCTGGCAC	TATTCCGGCTGCGACAAAAC
4958875	GGGATAACTTGCTGAAGGTC	GCTTAAGAGATTGGCTCTGG
2233308	AGACACCTTCCAGAGAGGA	AGAGCCAGGCCCTGGACAC
2233307	TCTTCCATCTGGTGAGTCTG	TCCTCTCTGGAAGGTGTCTG
2233306	CCACCCAGAACATCTCTGC	ATGGTGTGGCACCATGGCTG
2233305	CTAGCCTTCAGAGAGCTAAC	TCTGGCTTTGGCCTGCAACA
2233304	TGGCCCAGAGGGAAGCATCA	CCTGGATCCAGGGCCTAAC
2233303	TGGCCCAGAGGGAAGCATCA	CCTGGATCCAGGGCCTAAC
2233302	ACCAGGGCCCTCCAATCCAT	AAATGCCCAGGTAAGAGTGA
2287719	CTGCAGCTTCTCCACTTGCT	TCGTGAGCGCATGAATGAGG
2287720	AGGGAGGGTGAAGAGAGGGA	ATCTGTGACCCCAGCAGGAG
7727034	AGAGTAGTTCTGTGTCCCTC	TGGTTGATGGCTTTCAACGT
7727250	TTGGATCTGCTGGTTCTGAG	ATGTGAAACTACAGGCTAAG
7709800	TTGCTCATGACCTAGGCATC	CTTTATCTTCCTACTCCACC
3840312	GAAATGTGCTGGAAAGCAGG	CCTCTCCAGTTCAGTTTTGC
2287721	CCTTGAGTCCAGCTGCAATG	GGGTTACCCTTCCTAAGCTG
6875293	ATGGACATTACTGTACCGAC	TGGCCCAGCCCTTACGTTCT
3805434	AAATAAGGCCCCTGGTGTCC	GGGCATGGTCTTTTGCCTAG
2080982	CTGCCTCTTTAGCAGCTTTG	AACTTCTCCTCTGGGCCATA
2080983	CTGCCTCTTTAGCAGCTTTG	AACTTCTCCTCTGGGCCATA
2287722	CTGCCTCTTTAGCAGCTTTG	AACTTCTCCTCTGGGCCATA
2233301	CACAGGGCATGCCTGGTCTT	CCACTCCCCAAGGTTCAAAG
2233300	TGAACAAGCAGTGGGACCAG	AAGACCAGGCATGCCCTGTG
4958876	TTTTACCTTGCCAGGAAAGC	CAAAGAGGTGTGTGTGTTCC
2233299	CCAGCATCTTCACCTTCTTC	TTTTGGTGAAGGCTCCTCTG
2233298	TTTTGGTGAAGGCTCCTCTG	AGCATCTTCACCTTCTTCTC
2287723	AATTATCTCACCCAGTCTCG	TGGGAGGAATAGGGAGACAG
2161359	ACAGCTCTTCCTTACAAAAG	TTAGTGACCATTGGTGGATC
7734456	AATAGTCTAGAGAGTTCCCC	CCTGGGTTCTATAAGATGTTG
4292439	CCCTGGACAGGTCTCTTTAC	AGGAAGAGCATCTGGCTCGG

DbSNP rs#	Forward PCR primer	Reverse PCR primer
4958878	AGAGTTTACCCAGGACATGC	TAGAATGGAATACTAGTCCC
6862024	GTGCTGGGACTAGAGGATAG	TGTTGGCTAGTGTCTGTGTG
3834819	TGGGAACCACTCTCTAAGAC	GTGTTGAGTCGTTTGCTCAG
2233297	ATGAAGCCACAGTGACTACC	GTGGTAGAAGGTTCTAGCTG
2233296	AACCAAGGATGCTACAGAAG	ATGGAAGGGACAGGCAAGAA
2233295	TTGCCTGTCCCTTCCATCTT	ATCCCAGGGAGGAAAATTTG
2233294	TGGGATGGAGGTAAACAGAG	CAACTCATGCGATAGTGAGC
7713028	CTGGTGAAATACATCACCAC	TTGAGTAGCTGGGACTACAG
7713223	CTTTTGGATGGCCAAGGATG	CCAGCTTCAGTTTCTGAATC
7713567	AGCTGGTACCCACCAGATGA	ATCCTGTGAGTGGCCACAATT
888989	CAGGGACTTGATTGGAGTCT	ACTCACAGGATGGAGAGCAA
2233293	TCAGAGAAGGGGTGAGGAGT	AACCCAGAAATCAGTGCTGC
3749657	AGAAATCAGTGCTGCACACC	TAAGTTCAGGCCCCTCAGAGA
2233292	TGAACTTACCGCAGCCTCTC	CCAAGTTGGATAAGGGCCTG
2112635	TCCATCCCTTCTCCTTCAT	GGACTTCTCTGAGGAGATGG
871269	TTCCACAAACTTTGAGCTGG	TAATTAATTCCTGAGCTCCC
3792794	TCTGCTAGAAGGTAAGCTTG	AGTGTGTATGAAGTGCCACG
6579837	ATAGATCCTAGGCCCCTTAAA	AGGAAATAAACAAGGAGGGG
3805433	CTTAGCCTTGTGGGCTTTAG	AAGCCTCCGTTTTGCCATCT
5872186	TCCCGTATTTCCCCATTTCC	GCTTTTTGTTCAGGGTGATG
2233291	CCTTATCCTGTTCATCACCC	AATCCACTGGTACCATGGGG
2233290	CTGCAGGTGCAGCATCAGG	TACTCAGGCCTTGGTGAAGA
2233289	TCAGGCCTTGGTGAAGAGTG	ATCAGGTTGCCGTCCTCAC
4958435	AAGTTCTGCTCATCCTTCCC	GATCTCTGAGGCTCCCTGTA
4958880	TCTGGAGGAATGGCAATGAC	ATTTCCTCTTGTTAGAATGC
1422673	ATGCTTGTGTTCACTGGTTG	CATGCTAGTTAGCCAGACAG
2042234	GCACCTATTGCTCGCATAAC	TCTACCTTCTCCATTCTCCC
3805432	AAGGCCCAGGGCCCTGTAAGA	AACCTCCTTCCTGTGTGCTCC
3805431	AAATCACCTGTCTTACAGGG	TTTGGGTCTGTGGGCTTCCA
2233288	ATATGTGAGCGAGAAGCACC	ACAGAACCTGTTCAGATCCC
2233287	ATGACAGGCTTGTCGAGAAT	TTTTGAGTACACAGGGACCG
3815720	TGCTGGTTCAAGGTCAGCTA	TAATGTGAGGTCATATCCCC
3792792	TTCTCAGATCAGTTCACTCC	AACACAGATGGCAGCTGTTAC
3792791	GATTGCATAGTTCAGCATCC	CATTACCCGTGTAACCTCAG
2303018	CAAATGTCACAGCATCTCCC	TAAAACTTACACTGGATGGA
3792790	CATAAAAGCCCCATATCCCC	AGGGAGGGCCAAGTCTCAGT
4958436	GCCTTGGGGTCTTTATCAGC	ATATGCCAGCTCCAAGTGAC
2233286	ATGAATGGAAGGGTAGCCTG	ATCCTTCACAGTGAACTCCC
2233285	TTCCAAAAGCTCCCCTAGAG	ACGGGGAGAAGCAGCACAC
7732451	CATCTCTCGTGCCCCAGAAA	GCTGGAAGGCCCAGTTAATG
2233284	TACATGGTGCATAGAGACAG	ATATGCCTCTGCCCAAGTCA
1422674	TGCTGATAATTCCTCAGCAC	GTCCATTCACTCATCCATTC
3792789	GAGGATTCCTGATGAAACAG	TTCACTGGGGACCTCAGAAG
4562032	ATTTTACAAGGGAGAAACTG	CTGACTTTGCATCTTGGCTC
6865077	CTGACTTTGCATCTTGGCTC	ATTTTACAAGGGAGAAACTG
1559126	CACCATACTCACACAGCCAG	ATGGTGCTGCCCTACAAAGA
3792788	CCCTTCTCCCAAAGACTGAC	AGTGTGGGTAGCTGTCCTTT
1559127	ACTGTTTCTTAGTTCGGTGG	GGTGACTTGTTTTTAACCTG
3792786	TGAGGACAGAGGCAAGCAGA	AACAGTGTGGGGGCTGCACTT
6880110	TCAAGAACAGTGAAACAGAG	TGGTGGACAAGAACCAGGAC
6861227	CATGCCTTCATGTGGTGTTG	AGCAGTGTCCTGGTTCTTGT
3805430	TCCAGAGAGGGTTTTCCCAC	GGGAGAAAGAAAGGCAGTGG
1862364	AATCCCATGGCACACCACAG	TGTAGTTTGTGGAACCAGGC

DbSNP rs#	Forward PCR primer	Reverse PCR primer
4958881	GGACTGGAGGTCATCATAAC	TTCCACCCAAGGATGAAAGG
3792785	TTTGTTCTACCACTAACCAG	CTACATGGATGAGGAAGCAG
6869605	TTTGTCCAGCTTCTGTGTAC	AGGTGTGAGTTACCTAAGGG
6870205	GAATCATCAAGAAGGGACTG	ACCCCATTCTTTTTGATGGC
4246047	AAGCTGGTCTCAAACTCCTG	TGTTATTTAGGCTGAGCGTG
4958882	TTGTTGGTGGCTGATGAAGG	GCTCACTCATCTGGTTTAAG
3792784	ATGATTTTCCCATGACTGGC	AAACAGCCAGCTGCTGCTCA
3792783	AATCTGAAGAGTGACTGTCC	AAAGACCTTGGCGGCCACCAT
5872188	CCTTTGCCGACTTTGGTTTC	AGACTTGGAAGAGAATGCCC

TABLE 26

dbSNP rs#	Extend Primer	Term Mix
1478398	TGAGACAGGGCCTCACTCT	ACT
1478397	GTCTGAGGATCAGTAATAATAC	ACT
1160114	CTTAATTGCAATGCCTCTT	ACT
1160113	CTTGTTCCCTACCTAGC	ACT
1382323	CTGACCAGGGTAACAAC	ACT
1160112	TCTCCAATCACCGTTGT	ACG
7709870	CCTACAAGAAATATCAAGGC	ACG
7710643	GAGCATTACAGTTGTCAC	CGT
7730467	TTGCCACTAAATAAATCCAA	ACT
6579829	CATTCACTATGCTCTTTTTT	ACT
6579830	ATTCCCTGCTGAAGGTT	ACG
6579831	AATATGGGAGAGCCTTC	CGT
6896232	AATACACACAGGAGAGAAA	CGT
1351131	GAGAGCTTTCAGTGAAGT	ACT
1038074	GTGGGTTGTCCACATAT	ACG
1478396	CAAGACTCACTGCAATTTA	ACT
6880512	GGGCTTCAACATATGAAT	ACG
4958858	GAAGATGAAGGCCTGAG	ACT
4958431	CTTCAGGAAAATCCGGC	ACT
4958432	AAATACAGTCAGCCCCC	ACT
6898463	CTTCATTTCAGGTATTCTACT	ACT
4958859	TCAGAAGAGGAGAGCAC	ACT
4130064	CCTTCCAAAATTTCCCT	ACG
4130065	GGGAAATTTTGGAAGGT	ACT
4133119	GCCAAGTATCCTGTATCAA	ACT
4958860	AGGGCAACAATAAGGGA	ACT
4958861	AAGGAGCAGAAGCATGA	ACT
4437356	CTGAGCAGCCCTAGTGA	CGT
4958868	CTCCTGACCTCGTGATC	ACG
1478400	TGGGTGAAAAAATTGGTA	ACT
6889375	CTGCTGATGATGTAGGAAT	ACT
1600159	GAAGCTGAGAAGTCCCG	ACT
6875892	CCAATTCATTATAGGAAATTTTTT	CGT
4608909	GGTTTATCTCAGCATTGTTTAT	ACT
2345000	CTAGTCTCCTTGTTCTCTTA	ACT
4516840	TCCTTTTTCCACCTTGC	CGT
2054440	GTAAACATCCAAAAGCATTTC	ACT
707141	ACATCCTGAGATGAAGG	ACG

dbSNP rs#	Extend Primer	Term Mix
707142	CAGGCAGCAGCAGCACA	ACT
841236	ACCTTCTCTGAGGGTATTCTA	ACT
707143	CCCTTAGAGGTCATCTGGTC	ACG
707144	CCATACAAAATCCCCTTG	ACT
6869405	TGACTGGGACCCAGGCT	ACT
707145	GGCCCAGCAGCCAGAAG	ACG
707146	GAAAAGAAAGGCTAAGTGA	ACG
707148	ACTGAGGCCCAGAGAGG	CGT
707150	GTCTGCGCCACCTTCCC	CGT
5872184	GGAGACTCAATGCCAGG	ACT
3763015	CTGTGCTGGTGCTGGTG	ACG
2042235	ATCTCAGTTGTCCACCT	ACT
3763013	TACCCCTGGATTGCTAC	ACT
2042236	AGGCACCTAGGGTCACA	ACG
1946234	CACTGGAGATCACTAATC	CGT
1946235	CCTCAGTTTCCTCAGCT	ACT
1946236	ACACAGGGAGCAAATTT	CGT
8177402	CCAAACCACCTGGCTTA	ACT
8177403	GTCTTTGGGTCTTCATTT	ACG
8177404	TCCTCCCCATCATAAGG	ACT
8177405	TTCCTCCCCATCATAAG	ACT
8177406	CTCTGTCCCTACAGTCC	ACG
8177407	GCATTTCTTATTCCTATCGAG	ACT
8177408	GCTCCCTAGAACTGATG	ACT
8177409	GAGACTTCCATCAGTTCT	CGT
6888961	CCATTACAGCCAGGGCA	CGT
8177410	CTTTGGAGCCAAAAGAG	ACT
8177411	GCGGCCAAGCCGAGACC	ACT
8177412	GATTGGCTGCAAGGGTC	ACG
8177413	TCCTGCCTGCTTTCCCT	ACT
870407	GACCCTCGAGGTGGCAG	ACG
870406	AGATGTAGCAAGGCGAC	ACG
6873202	CAGAGATCTGTGCTAGAA	ACT
8177414	TGTTCTCTGAAATTTCCTC	ACG
8177415	AGCAGGTGTGTGGCCCA	ACG
3805435	TACTGGGGTGGGCTCTG	ACG
8177416	TGCATGTTGGGAAGTTG	ACT
3792799	CGGAATCCTAGACTCATAA	ACT
3792798	ATAGACAGGCCAGCACC	ACT
3828599	CAACCTTCAGTTTTGGAA	ACT
8177417	ACGTTCTCCCCACTAGC	ACT
3792797	ATCCTTCCCCAGAGTGC	ACT
8177418	CTCACCCTAAAACTTTTCTA	ACT
8177419	CTGCTCCCAAGTTCTTA	ACT
8177420	ACATGCCCCATAACCCT	ACT
8177421	TCCCCAGTCCAACTTCA	ACT
4958872	GCAGTTACTAGGATCCC	ACT
3792796	TTGCTGTGTCTCTGATG	ACT
8177422	CATCAGAGACACAGCAA	ACT
8177423	CAGAGACACAGCAAAGC	ACG
4958434	TCTCCTCTCCCGTCTTAT	ACG

dbSNP rs#	Extend Primer	Term Mix
8177424	TGGATGATGGGACTCAG	ACG
8177425	GAACGAAATCTCATGTCAA	ACT
8177426	AGCAGATTCCCACAACC	ACT
8177427	GACTTGCTCAGGGCCAC	ACT
8177429	CCTAATAATGGGAACTTGTAAA	ACT
6889737	GGCAGCTCTTCTGCACT	CGT
3792795	TTCAAGGTTTCTCCTTTC	ACG
8177430	ATGAGCCTACTCTGCCC	ACT
8177431	CATGGTTGACCTGGTTG	ACT
4958873	ACAGGGAGTTAATGGCA	ACT
8177432	TGTTGCTTTTCCCTACC	ACT
8177433	GCTGTTGTTACCAAATAGG	ACT
8177434	GAAGCTGTTGTTACCAAA	ACG
8177435	GAATCACCATGTCCATAC	ACT
3763011	AAGCACTTAATATTAAGTACCC	ACT
8177436	TGTGAAGATGATTATATAAGCC	ACG
8177437	AAGCTCCATCTTGCTGA	ACT
4958874	TGGGGCTGAGGGAAAAT	ACT
8177439	TTTCACTTCCGGGAATT	ACG
8177440	TCCCGGAAGTGAAAGGA	ACG
8177441	TCAGTGTCCCCTGGTCT	ACT
8177442	CTCAGTGTCCCCTGGTC	CGT
8177443	GAGGGTTGTAACCTCAC	ACG
869975	GGGCCTCAGTAGTTCCAGC	ACT
869976	CCAAAGAGAAAGAGCAGA	ACG
8177444	GATAAATGTCCACCATGA	CGT
8177445	CTGGAAATTAGGGACAA	ACT
7721469	ACCCTGACTAGGGTCTC	CGT
8177446	GCTGTGGCTCTAGAAGA	ACT
7704191	ATAGCCTGGCCCTGAGA	ACT
8177447	AGTTCTAGAGCAGGGAT	ACT
11548	CACACTATCTACCCATCA	ACG
2230303	GTACACAGAACTGTATGC	CGT
7722386	ACCCACACCCACATGCC	ACG
8177448	CATAGGTAGACACGTGG	ACG
8177449	AAGACACATAGGTAGACAC	ACT
2070593	GCATGGGTGTACAGCCAC	ACT
8177450	CCAGAAAGACACATAGG	ACG
8177451	CCCAGAAAGACACATAG	ACT
8177452	TGTCACATCTGCCTTGG	ACG
8177453	CCTGCCCAGGGGCCTTA	ACT
8177454	TCTTTTCTCATCCTCCC	ACT
3763010	AAAGGTAATTGCGGTTTT	ACG
8177455	GGTGGCATCTTCATGAG	ACT
8177456	TGAGGCTATAGTGTGCC	ACT
736775	TCCCTGAGGGTAGGGCA	ACT
2277940	CATGTTCATTTATGCGCT	ACT
8177458	GCCATGCCAGCCACGTC	ACG
8177834	CCTGGAGGCTTCTGCAA	ACG
3924	CTCAGTTCAGGGACTGGT	ACT
2233312	TGATCTCAGATTGCCAA	ACT
2233311	CAGAGCCAGCTGATCTCA	ACT

dbSNP rs#	Extend Primer	Term Mix
2233310	CCAAGAGCAGAAACTAAC	ACT
2233309	TGCTATTGTAATTTTTGGGT	ACT
4958875	CTGAAGGTCATCTAGCAA	ACT
2233308	CTTCTGAACTAAACAGCAC	ACG
2233307	GCTCCAGGGCACACACA	ACT
2233306	CTACCCCTACGCCTACC	ACG
2233305	GATGAGATGGGTGTCCT	ACT
2233304	TCAGCCGGTCAGTCCTC	ACT
2233303	ATCAGCCGGTCAGTCCT	ACG
2233302	TCCATGCCCCCTCTCCC	ACT
2287719	CTCCACTTGCTTCTTCA	ACT
2287720	TTCTCCTCAGGCCCAGA	ACG
7727034	ACATTCTGGGCTTCAAG	ACT
7727250	AGAGACCCTACAAACTC	ACG
7709800	CCTAGGCATCTCCCTGT	ACG
3840312	CTAGAGCCGTTCCCACC	CGT
2287721	CCCATCACTGGCACCCC	ACG
6875293	GAGCATTGTGAAGTGATG	ACT
3805434	TGCTGACGGGAGGAACT	ACT
2080982	AACCTTGGGGAGTGGCC	CGT
2080983	TGAACCTTGGGGAGTGG	CGT
2287722	GCAGCTTTGAACCTTGG	ACT
2233301	TGCCTGGTCTTCATCTC	ACT
2233300	GGTAATGTGGGGTTCCT	ACT
4958876	CTAAGACATGGAAACTACAT	CGT
2233299	TCACCTTCTTCTCGGCTGC	ACT
2233298	CCCCAGGCTAGTGTGAC	ACT
2287723	AAGACCCCTCACCCAAG	ACT
2161359	CATTCCATTACTACAGTAATAACT	ACT
7734458	CCCTTCCTTTACTTCCC	ACT
4292439	CCCTTTAATCTCCACTC	ACT
4958878	CAGGACATGCAAATCTATT	CGT
6862024	CTAGAGGATAGGGGAGT	ACT
3834819	CTTGACAAACTGGAATGT	ACT
2233297	ATGGAGGTCAGGCCAAG	ACG
2233296	ACATACCTGCTGCTGTC	ACG
2233295	AGCCTGGCCGCCCAGAC	ACT
2233294	CCCTGAGTTAAGAAACCT	CGT
7713028	TACATCACCACCCCTCC	ACG
7713223	GGGGTTTTTATAGCCCTA	ACT
7713567	ACCAGATGACTCATCAC	ACG
888889	GATTGGAGTCTTACAACA	ACT
2233293	GGGGCTGCAGGAGGAGG	ACT
3749657	CCTCCTCCTGCAGCCCC	CGT
2233292	TCTCGGCAGCCTGATCC	ACG
2112635	CCCCAGCTGACTTCTAC	ACG
871269	CTGGCAGTGAACAAGAG	ACT
3792794	TGTTGTATTCACTGTTGC	ACT
6579837	AGGGGTCCTGCCAACCT	CGT
3805433	TGGGCTTTAGCAGCGGG	ACT
5872186	CCTCATCCCTCCCCACA	CGT
2233291	GTCTGCCCACAACACAG	ACT

dbSNP rs#	Extend Primer	Term Mix
2233290	GTTGCCGTCCTCACGGG	ACT
2233289	CCCTTTCCCTCTCCAGG	ACG
4958435	ATGGGAATCACAGGACA	ACT
4958880	GAATGGCAATGACTATAACC	CGT
1422673	TGTCAGTGCCCAGTGCA	ACT
2042234	CTCGCATAACAACAATTAAAG	ACT
3805432	CCTGTAAGACAGGTGATTT	ACT
3805431	GGGCCCTGGGCCTTCCT	ACT
2233288	CCCCAAGTGACTCCAGG	ACT
2233287	AATCCTGCTTTGTGATCT	ACG
3815720	AAGGTCAGCTAGTCCCA	ACG
3792792	GGGAAGGCCAGCACAGG	ACT
3792791	AGGTGCAGGGAAAGAAG	ACT
2303018	CAGCATCTCCCACAGCC	ACG
3792790	GCTTGGGAAGGTCATCA	CGT
4958436	TGTGTGCCAGGCATCGC	ACT
2233286	GATAAACGAGAGAATGTGG	ACT
2233285	GGAAGCAAAAGCATTTACT	ACG
7732451	GTGCCCCAGAAAGAGGAG	ACT
2233284	GTGAGGCAGGAGCCAGC	ACT
1422674	CACCCAGACTGGTGCCT	CGT
3792789	CCTTGGGAGGAAGGTGC	ACG
4562032	TAACTCAGCCACGATGA	CGT
6865077	GCATCTTGGCTCTTATTG	ACG
1559126	ACAGCCAGGACACAGAG	ACT
3792788	CAGATACAAGATGAATACACC	ACT
1559127	TCGGTGGAAACATCTGC	ACT
3792786	GACCCCCAATGTCTGCC	ACT
6880110	GCCACCTTGCATGACAA	ACT
6861227	GAGTTCCATTTAGGGTG	CGT
3805430	CAATAGGATATTTCTCCTGC	ACT
1862364	CCCCACCACATCTTCTC	ACT
4958881	GCAATGTGATATCATGGC	ACT
3792785	TCTCAGGCAAATGACTT	ACT
6869605	CTCTAGATTCCTAGATAGGG	ACT
6870205	ACAAACCACCATTCATTC	ACT
4246047	TGCTCTGCCTCCCAAAG	CGT
4958882	TGATGAAGGAGAAATTTCAA	ACT
3792784	TCACTCTTCAGATTGGAA	ACG
3792783	AAATCATCAAGAACTTCCC	ACT
5872188	GAATGAAAATGTTTCACTCT	ACT

Genetic Analysis

[0254] Allelotyping results are shown for female cases and controls in Table 27. The allele frequency for the A2 allele is noted in the fifth and sixth columns for control pools and case pools, respectively, where "AF" is allele frequency. Some SNPs do not have an allele frequency disclosed because of failed assays.

TABLE 27

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
1478398	231	150385031	A/G	A=0.28 G=0.72	A=0.34 G=0.66	0.0334	1.31	A
1478397	330	150385130	T/C	T=0.61 C=0.39	T=0.67 C=0.33	0.0621	1.29	T
1160114	582	150385382	G/C	G=0.96 C=0.04	G=0.92 C=0.08	0.0034	0.44	C
1160113	589	150385389	A/G	A=0.96 G=0.04	A=0.93 G=0.07	0.0169	0.51	G
1382323	1060	150385860	A/G	A=0.67 G=0.33	A=0.63 G=0.37	0.1288	0.83	G
1160112	1066	150385866	G/A	G=0.91 A=0.09	G=0.85 A=0.15	0.0025	0.57	A
7709870	1311	150386111	G/A	G=0.11 A=0.89	G=0.12 A=0.88	0.5796	1.11	G
7710643	1556	150386356	G/T	G=0.87 T=0.13	G=0.86 T=0.14	0.7100	0.93	T
7730467	1655	150386455	T/C	T=1.00 C=0.00	T=1.00 C=0.00			
6579829	1692	150386492	A/C	A=0.92 C=0.08	A=0.89 C=0.11	0.1423	0.74	C
6579830	1802	150386602	G/A	G=0.91 A=0.09	G=0.89 A=0.11	0.4377	0.86	A
6579831	2061	150386861	T/A	T=0.31 A=0.69	T=0.32 A=0.68	0.7948	1.04	T
6896232	2112	150386912	T/A	T=0.90 A=0.10	T=0.89 A=0.11	0.7880	0.94	A
1351131	2153	150386953	T/C	T=0.51 C=0.49	T=0.49 C=0.51	0.4152	0.91	C
1038074	2667	150387467	C/T	C=0.23 T=0.77	C=0.27 T=0.73	0.1062	1.25	C
1478396	3115	150387915	T/C	T=0.92 C=0.08	T=0.90 C=0.10	0.1123	0.71	C
6880512	3186	150387986	G/A	G=0.64 A=0.36	G=0.60 A=0.40	0.2313	0.86	A
4958858	5621	150390421	T/C	T=0.06 C=0.94	T=0.13 C=0.87	~0.00001	2.54	T
4958431	5735	150390535	T/G	T=0.98 G=0.02	T=0.95 G=0.05	0.0132	0.44	G
4958432	5829	150390629	G/C	G=0.86 C=0.14	G=0.85 C=0.15	0.4357	0.87	C
6898463	6658	150391458	A/C	A=0.86 C=0.14	A=0.82 C=0.18	0.0341	0.71	C
4958859	7901	150392701	G/C	G=0.95 C=0.05	G=0.90 C=0.10	0.0030	0.48	C
4130064	11447	150396247	G/A	G=0.26 A=0.74	G=0.30 A=0.70	0.1514	1.21	G

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
4130065	11466	150396266	A/G	A=0.16	A=			
				G=0.84	G=			
4133119	11984	150396784	T/C	T=0.95	T=0.94	0.2216	0.72	C
				C=0.05	C=0.06			
4958860	15803	150400603	T/G	T=0.10	T=0.17	0.0163	1.88	T
				G=0.90	G=0.83			
4958861	16257	150401057	T/G	T=0.13	T=0.17	0.0581	1.38	T
				G=0.87	G=0.83			
4437356	17604	150402404	C/A	C=0.10	C=			
				A=0.90	A=			
4958868	19762	150404562	C/T	C=0.55	C=0.53	0.6623	0.94	T
				T=0.45	T=0.47			
1478400	22367	150407167	A/G	A=0.96	A=0.94	0.0584	0.57	G
				G=0.04	G=0.06			
6889375	22709	150407509	A/G	A=0.59	A=0.61	0.5378	1.09	A
				G=0.41	G=0.39			
1600159	23631	150408431	G/C	G=0.62	G=0.61	0.7340	0.98	C
				C=0.38	C=0.39			
6875892	23686	150408486	T/A	T=1.00	T=1.00			
				A=0.00	A=0.00			
4608909	25599	150410399	T/C	T=0.98	T=0.92	0.0008	0.27	C
				C=0.02	C=0.08			
2345000	26973	150411773	A/C	A=0.57	A=0.55	0.5708	0.93	C
				C=0.43	C=0.45			
4516840	28457	150413257	G/T	G=0.00	G=0.00			
				T=1.00	T=1.00			
2054440	28669	150413469	A/G	A=0.62	A=0.66	0.1835	1.18	A
				G=0.38	G=0.34			
707141	29908	150414708	C/T	C=0.45	C=0.46	0.6490	1.06	C
				T=0.55	T=0.54			
707142	30105	150414905	A/G	A=0.54	A=0.53	0.7349	0.98	G
				G=0.46	G=0.47			
841236	30711	150415511	A/G	A=0.55	A=0.54	0.7731	0.97	G
				G=0.45	G=0.46			
707143	30851	150415651	G/A	G=0.64	G=0.66	0.6049	1.07	G
				A=0.36	A=0.34			
707144	31203	150416003	T/C	T=0.69	T=0.75	0.0163	1.38	T
				C=0.31	C=0.25			
6889405	31446	150416246	A/C	A=0.08	A=0.09	0.5734	1.13	A
				C=0.92	C=0.91			
707145	31638	150416438	C/T	C=0.62	C=0.61	0.8223	0.97	T
				T=0.38	T=0.39			
707146	33064	150417864	C/T	C=0.53	C=0.48	0.1214	0.83	T
				T=0.47	T=0.52			
707148	33958	150418758	C/A	C=0.46	C=0.48	0.6249	1.08	C
				A=0.54	A=0.52			
707150	35182	150419982	A/T	A=0.67	A=0.67	0.9445	0.99	A
				T=0.33	T=0.33			
5872184	38332	150423132	-/C	-=0.00	-=0.00			

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
				C=1.00	C=1.00			
3783015	40875	150425675	G/A	G=0.61	G=0.60	0.7976	0.97	A
				A=0.39	A=0.40			
2042235	41624	150426424	T/C	T=0.13	T=0.20	0.0014	1.70	T
				C=0.87	C=0.80			
3783013	41671	150426471	A/G	A=0.97	A=0.82	~0.00001	0.14	G
				G=0.03	G=0.18			
2042236	41825	150426625	G/A	G=0.90	G=0.86	0.0420	0.69	A
				A=0.10	A=0.14			
1946234	42920	150427720	C/A	C=0.00	C=0.00			
				A=1.00	A=1.00			
1946235	42935	150427735	T/C	T=	T=0.87			
				C=	C=0.13			
1946236	43001	150427801	T/A	T=0.19	T=0.20	0.5482	1.10	T
				A=0.81	A=0.80			
8177402	43012	150427812	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
8177403	43203	150428003	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
8177404	43294	150428094	T/C	T=0.97	T=0.94	0.0363	0.52	C
				C=0.03	C=0.06			
8177405	43295	150428095	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
8177406	43344	150428144	C/T	C=0.09	C=0.11	0.1222	1.36	C
				T=0.91	T=0.89			
8177407	43509	150428309	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
8177408	43549	150428349	G/C	G=0.00	G=0.00			
				C=1.00	C=1.00			
8177409	43560	150428360	T/A	T=0.23	T=0.28	0.0779	1.29	T
				A=0.77	A=0.72			
6888961	43578	150428378	A/T	A=0.08	A=0.07	0.3178	0.79	T
				T=0.92	T=0.93			
8177410	43640	150428440	A/G	A=	A=0.00			
				G=	G=1.00			
8177411	43792	150428592	G/C	G=1.00	G=1.00			
				C=0.00	C=0.00			
8177412	43797	150428597	C/T	C=0.15	C=0.18	0.0943	1.31	C
				T=0.85	T=0.82			
8177413	43964	150428764	C/G	C=	C=0.01			
				G=	G=0.99			
870407	44297	150429097	C/T	C=0.07	C=0.11	0.0641	1.49	C
				T=0.93	T=0.89			
870408	44311	150429111	C/T	C=0.89	C=0.88	0.5675	0.87	T
				T=0.11	T=0.12			
6873202	44588	150429388	A/G	A=1.00	A=1.00			
				G=0.00	G=0.00			
8177414	44775	150429575	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
8177415	44921	150429721	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
3805435	45006	150429806	G/A	G=0.10	G=0.07	0.1318	0.71	A
				A=0.90	A=0.93			
8177416	45098	150429898	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
3792799	45185	150429985	C/G	C=0.00	C=0.00			
				G=1.00	G=1.00			
3792798	45475	150430275	T/C	T=0.05	T=0.10	0.0906	2.06	T
				C=0.95	C=0.90			
3828599	45506	150430306	T/C	T=0.55	T=0.55	0.9993	1.00	T
				C=0.45	C=0.45			
8177417	45543	150430343	G/C	G=1.00	G=1.00			
				C=0.00	C=0.00			
3792797	45601	150430401	T/G	T=0.46	T=0.52	0.0520	1.27	T
				G=0.54	G=0.48			
8177418	45652	150430452	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
8177419	45756	150430556	A/-	A=0.00	A=0.00			
				-=1.00	-=1.00			
8177420	45826	150430626	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
8177421	45974	150430774	C/G	C=0.07	C=0.02			
				G=0.93	G=0.98			
4958872	46044	150430844	T/C	T=0.81	T=0.81	0.9167	0.98	T
				C=0.19	C=0.19			
3792796	46200	150431000	C/G	C=0.40	C=0.41	0.5382	1.08	C
				G=0.60	G=0.59			
8177422	46218	150431018	A/G	A=1.00	A=1.00			
				G=0.00	G=0.00			
8177423	46221	150431021	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
4958434	46280	150431080	C/T	C=0.86	C=0.83	0.1864	0.79	T
				T=0.14	T=0.17			
8177424	46330	150431138	/GAGT CCTGG	-=1.00 GAGTCCTG	-=0.98 GAGTCCTG			
				G=0.00	G=0.02			
8177425	46583	150431383	T/C	T=0.02	T=0.03	0.5080	1.30	T
				C=0.98	C=0.97			
8177426	46650	150431450	A/G	A=0.31	A=0.36	0.0447	1.29	A
				G=0.69	G=0.64			
8177427	46721	150431521	A/G	A=0.26	A=0.32	0.0296	1.33	A
				G=0.74	G=0.68			
8177429	46808	150431608	G/C	G=0.91	G=0.95	0.0132	1.89	G
				C=0.09	C=0.05			
6889737	47242	150432042	C/A	C=1.00	C=1.00			
				A=0.00	A=0.00			
3792795	47512	150432312	G/A	G=0.89	G=0.97	0.0000	3.53	G
				A=0.11	A=0.03			

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
8177430	47600	150432400	T/C	T=0.01	T=0.01			
				C=0.99	C=0.99			
8177431	47706	150432506	A/G	A=0.71	A=0.70	0.6316	0.94	G
				G=0.29	G=0.30			
4958873	47806	150432606	A/G	A=0.31	A=0.38	0.0279	1.32	A
				G=0.69	G=0.62			
8177432	47978	150432778	T/G	T=1.00	T=1.00			
				G=0.00	G=0.00			
8177433	48021	150432821	T/C	T=0.26	T=0.23	0.2375	0.85	C
				C=0.74	C=0.77			
8177434	48025	150432825	G/A	G=0.00	G=0.00			
				A=1.00	A=1.00			
8177435	48093	150432893	T/G	T=0.81	T=0.79	0.4469	0.89	G
				G=0.19	G=0.21			
3763011	48413	150433213	T/C	T=0.01	T=0.02			
				C=0.99	C=0.98			
8177436	48933	150433733	C/T	C=0.00	C=0.00			
				T=1.00	T=1.00			
8177437	49097	150433897	C/G	C=0.17	C=0.07	~0.00001	0.38	G
				G=0.83	G=0.93			
4958874	49105	150433905	T/C	T=0.44	T=0.49	0.0880	1.24	T
				C=0.56	C=0.51			
8177439	49570	150434370	G/A	G=	G=1.00			
				A=	A=0.00			
8177440	49591	150434391	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
8177441	49704	150434504	G/C	G=1.00	G=1.00			
				C=0.00	C=0.00			
8177442	49705	150434505	A/T	A=1.00	A=1.00			
				T=0.00	T=0.00			
8177443	49798	150434598	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
869975	50082	150434882	A/G	A=0.37	A=0.25	~0.00001	0.57	G
				G=0.63	G=0.75			
869976	50147	150434947	G/A	G=0.00	G=0.00			
				A=1.00	A=1.00			
8177444	50356	150435156	A/T	A=1.00	A=1.00			
				T=0.00	T=0.00			
8177445	50725	150435525	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
7721469	50968	150435768	C/A	C=0.00	C=0.00			
				A=1.00	A=1.00			
8177446	51029	150435829	A/C	A=1.00	A=1.00			
				C=0.00	C=0.00			
7704191	51086	150435886	T/C	T=1.00	T=1.00			
				C=0.00	C=0.00			
8177447	51166	150435966	T/C	T=0.27	T=0.35	0.0030	1.50	T
				C=0.73	C=0.65			
11548	51493	150436293	C/T	C=0.90	C=0.95	0.0002	2.40	C

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
				T=0.10	T=0.05			
2230303	51539	150436339	G/T	G=0.00	G=0.01			
				T=1.00	T=0.99			
7722386	51562	150436362	G/A	G=0.00	G=0.00			
				A=1.00	A=1.00			
8177448	51645	150436445	G/A	G=0.99	G=0.99			
				A=0.01	A=0.01			
8177449	51649	150436449	T/C	T=0.17	T=0.14	0.2009	0.80	C
				C=0.83	C=0.86			
2070593	51650	150436450	T/C	T=0.31	T=0.28	0.2540	0.86	C
				C=0.69	C=0.72			
8177450	51656	150436456	G/A	G=0.00	G=0.00			
				A=1.00	A=1.00			
8177451	51657	150436457	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
8177452	52009	150436809	G/A	G=0.00	G=0.00			
				A=1.00	A=1.00			
8177453	52143	150436943	A/C	A=0.00	A=0.00			
				C=1.00	C=1.00			
8177454	52349	150437149	G/C	G=0.99	G=0.98			
				C=0.01	C=0.02			
3763010	52421	150437221	C/T	C=0.76	C=0.80	0.1337	1.30	C
				T=0.24	T=0.20			
8177455	52532	150437332	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
8177456	52682	150437482	A/G	A=0.45	A=0.50	0.1098	1.22	A
				G=0.55	G=0.50			
736775	53058	150437858	T/C	T=0.56	T=0.59	0.3945	1.12	T
				C=0.44	C=0.41			
2277940	53187	150437987	T/C	T=0.92	T=0.97	**0.0004**	2.75	T
				C=0.08	C=0.03			
8177458	53377	150438177	G/A	G=0.98	G=0.98	0.9445	1.03	G
				A=0.02	A=0.02			
8177834	53699	150438499	G/A	G=0.93	G=0.89	**0.0113**	0.58	A
				A=0.07	A=0.11			
3924	53845	150438645	A/G	A=0.75	A=0.74	0.7350	0.95	G
				G=0.25	G=0.26			
2233312	53920	150438720	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
2233311	53929	150438729	T/G	T=0.15	T=0.20	**0.0106**	1.49	T
				G=0.85	G=0.80			
2233310	55473	150440273	T/C	T=0.00	T=0.00			T
				C=1.00	C=1.00			
2233309	55690	150440490	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
4958875	55850	150440650	A/G	A=0.56	A=0.53	0.2131	0.86	G
				G=0.44	G=0.47			
2233308	56761	150441561	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
2233307	56840	150441640	C/G	C=0.00	C=0.00			
				G=1.00	G=1.00			
2233306	57000	150441800	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
2233305	57116	150441916	T/G	T=	T=0.27			
				G=	G=0.73			
2233304	58419	150443219	T/G	T=0.00	T=0.01			
				G=1.00	G=0.99			
2233303	58420	150443220	C/T	C=1.00	C=1.00			
				T=0.00	T=0.00			
2233302	58808	150443608	G/C	G=0.98	G=0.87	~0.00001	0.14	C
				C=0.02	C=0.13			
2287719	58906	150443706	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
2287720	59048	150443848	C/T	C=0.39	C=0.42	0.2852	1.14	C
				T=0.61	T=0.58			
7727034	59187	150443987	C/G	C=0.24	C=0.30	0.0374	1.36	C
				G=0.76	G=0.70			
7727250	59361	150444161	C/T	C=0.33	C=0.43	0.0003	1.57	C
				T=0.67	T=0.57			
7709800	61218	150446018	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
3840312	61700	150446500	G/-	G=0.54	G=0.50	0.1346	0.84	-
				-=0.46	-=0.50			
2287721	62290	150447090	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
6875293	62596	150447396	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
3805434	64049	150448849	G/C	G=0.93	G=0.87	0.0009	0.51	C
				C=0.07	C=0.13			
2080982	66077	150450877	G/T	G=0.02	G=0.01			
				T=0.98	T=0.99			
2080983	66079	150450879	G/T	G=1.00	G=1.00			
				T=0.00	T=0.00			
2287722	66086	150450886	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
2233301	66115	150450915	T/G	T=0.00	T=0.00			
				G=1.00	G=1.00			
2233300	66150	150450950	C/G	C=0.00	C=0.00			
				G=1.00	G=1.00			
4958876	66475	150451275	C/A	C=	C=0.95			
				A=	A=0.05			
2233299	69177	150453977	A/G	A=0.48	A=0.49	0.7917	1.03	A
				G=0.52	G=0.51			
2233298	69210	150454010	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
2287723	69312	150454112	T/G	T=1.00	T=1.00			
				G=0.00	G=0.00			
2161359	70244	150455044	A/G	A=0.53	A=0.51	0.5813	0.94	G

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
				G=0.47	G=0.49			
7734456	70882	150455682	G/C	G=0.53	G=0.60	0.0188	1.32	G
				C=0.47	C=0.40			
4292439	71905	150456705	T/C	T=0.87	T=0.84	0.1343	0.77	C
				C=0.13	C=0.16			
4958878	72294	150457094	A/T	A=	A=0.12			
				T=	T=0.88			
6862024	72581	150457381	A/G	A=0.80	A=0.78	0.4512	0.89	G
				G=0.20	G=0.22			
3834819	72786	150457589	-/CA	-=0.00	-=0.00			
				CA=1.00	CA=1.00			
2233297	72950	150457750	G/A	G=0.04	G=0.05	0.3215	1.32	G
				A=0.96	A=0.95			
2233296	73106	150457906	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
2233295	73162	150457962	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
2233294	73273	150458073	G/T	G=0.24	G=0.23	0.7059	0.95	T
				T=0.76	T=0.77			
7713028	74131	150458931	G/A	G=0.9	G=0.85	0.0238	0.66	A
				A=0.1	A=0.15			
7713223	74406	150459206	T/C	T=0.93	T=0.88	0.0047	0.56	C
				C=0.07	C=0.12			
7713567	74865	150459465	C/T	C=0.61	C=0.63	0.4135	1.10	C
				T=0.39	T=0.37			
888989	74740	150459540	T/C	T=0.90	T=0.86	0.0352	0.67	C
				C=0.10	C=0.14			
2233293	75382	150460182	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
3749657	75400	150460200	G/T	G=1.00	G=1.00			
				T=0.00	T=0.00			
2233292	75460	150460260	G/A	G=	G=1.00			
				A=	A=0.00			
2112635	75863	150460663	C/T	C=0.35	C=0.36	0.7381	1.04	C
				T=0.65	T=0.64			
871269	76098	150460898	T/C	T=0.47	T=0.42	0.0796	0.81	C
				C=0.53	C=0.58			
3792794	78432	150463232	A/G	A=0.21	A=0.10	~0.00001	0.41	G
				G=0.79	G=0.90			
6579837	78604	150463404	G/T	G=0.89	G=0.91	0.1630	1.33	G
				T=0.11	T=0.09			
3805433	79190	150463990	C/G	C=0.28	C=0.28	0.9484	0.99	C
				G=0.72	G=0.72			
5872186	79870	150464670	-/A	-=0.33	-=0.35	0.5526	1.08	-
				A=0.67	A=0.65			
2233291	79928	150464728	G/C	G=1.00	G=1.00			
				C=0.00	C=0.00			
2233290	80213	150465013	G/C	G=0.14	G=0.13	0.8704	0.97	C
				C=0.86	C=0.87			

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
2233289	80227	150465027	C/T	C=1.00	C=0.99			
				T=0.00	T=0.010			
4958435	81994	150466794	T/G	T=0.68	T=0.63	0.0549	0.79	G
				G=0.32	G=0.37			
4958880	82187	150466987	C/A	C=0.75	C=0.84	0.0002	1.76	C
				A=0.25	A=0.16			
1422673	82698	150467498	T/C	T=0.45	T=0.32	~0.00001	0.59	C
				C=0.55	C=0.68			
2042234	82841	150467641	A/G	A=0.91	A=0.93	0.1485	1.37	A
				G=0.09	G=0.07			
3805432	83214	150468014	A/G	A=0.04	A=0.07	0.0188	1.93	A
				G=0.96	G=0.93			
3805431	83249	150468049	T/C	T=0.06	T=0.02			
				C=0.94	C=0.98			
2233288	83485	150468285	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
2233287	83807	150468607	C/T	C=0.90	C=0.92	0.2555	1.32	C
				T=0.10	T=0.08			
3815720	83907	150468707	G/A	G=0.99	G=0.97			
				A=0.01	A=0.03			
3792792	84216	150469016	A/G	A=1.00	A=0.99			
				G=0.00	G=0.01			
3792791	84656	150469456	A/G	A=0.00	A=0.00			
				G=1.00	G=1.00			
2303018	85448	150470248	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
3792790	85881	150470681	G/T	G=0.49	G=0.49	0.9686	1.00	G
				T=0.51	T=0.51			
4958436	86539	150471339	T/C	T=0.88	T=0.92	0.0083	1.76	T
				C=0.12	C=0.08			
2233286	86796	150471596	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
2233285	87057	150471857	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
7732451	87922	150472722	T/C	T=0.90	T=0.92	0.2349	1.29	T
				C=0.10	C=0.08			
2233284	88098	150472898	T/C	T=0.00	T=0.00			
				C=1.00	C=1.00			
1422674	89319	150474119	G/T	G=	G=			
				T=	T=			
3792789	89678	150474478	C/T	C=0.42	C=0.42	0.9873	1.00	C
				T=0.58	T=0.58			
4562032	90026	150474826	C/A	C=	C=1.00			
				A=	A=0.00			
6865077	90033	150474833	G/A	G=1.00	G=1.00			
				A=0.00	A=0.00			
1559126	90114	150474914	C/G	C=1.00	C=1.00			
				G=0.00	G=0.00			
3792788	90326	150475126	T/C	T=0.00	T=0.00			

dbSNP rs#	Position in SEQ ID NO: 5	Chromo-some Position	A1/A2 Allele	Control AF (High BMD)	Case AF (Low BMD)	p-Value	OR	Low BMD Associated Allele
				C=1.00	C=1.00			
1559127	90463	150475263	T/C	T=0.94	T=0.94	0.6080	1.14	T
				C=0.06	C=0.06			
3792786	90548	150475348	A/G	A=0.98	A=0.99			
				G=0.02	G=0.01			
6880110	90800	150475600	A/G	A=0.91	A=0.92	0.5433	1.14	A
				G=0.09	G=0.08			
6861227	90838	150475638	G/T	G=0.26	G=0.23	0.1843	0.83	T
				T=0.74	T=0.77			
3805430	91400	150476200	C/G	C=0.02	C=0.05			
				G=0.98	G=0.95			
1862364	92086	150476886	A/G	A=0.91	A=0.92	0.5575	1.13	A
				G=0.09	G=0.08			
4958881	93946	150478746	T/C	T=0.91	T=0.91	0.9701	1.01	T
				C=0.09	C=0.09			
3792785	95360	150480160	A/G	A=0.92	A=0.93	0.5448	1.16	A
				G=0.08	G=0.07			
6869605	96576	150481376	A/C	A=0.90	A=0.89	0.7189	0.93	C
				C=0.10	C=0.11			
6870205	96721	150481521	A/G	A=0.00	A=0.07			
				G=1.00	G=0.93			
4246047	98316	150483116	T/A	T=0.94	T=0.96	0.2613	1.37	T
				A=0.06	A=0.04			
4958882	98497	150483297	C/G	C=0.88	C=0.89	0.7723	1.06	C
				G=0.12	G=0.11			
3792784	99382	150484182	C/T	C=0.00	C=0.04			
				T=1.00	T=0.96			
3792783	99442	150484242	T/C	T=0.85	T=0.87	0.2391	1.22	T
				C=0.15	C=0.13			
5872188	99764	150484565	-/AG	-=0.90	-=0.91	0.5908	1.11	-
				AG=0.10	AG=0.09			

[0255] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 4. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 4 can be determined by consulting Table 27. For example, the left-most X on the left graph is at position 150385031. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0256] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are

drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0257] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is place at the 3' end of each gene to show the direction of transcription.

Example 10

In Vitro Production of Target Polypeptides

[0258] cDNA is cloned into a pIVEX 2.3-MCS vector (Roche Biochem) using a directional cloning method. A cDNA insert is prepared using PCR with forward and reverse primers having 5' restriction site tags (in frame) and 5-6 additional nucleotides in addition to 3' gene-specific portions, the latter of which is typically about twenty to about twenty-five base pairs in length. A Sal I restriction site is introduced by the forward primer and a Sma I restriction site is introduced by the reverse primer. The ends of PCR products are cut with the corresponding restriction enzymes (*i.e.*, Sal I and Sma I) and the products are gel-purified. The pIVEX 2.3-MCS vector is linearized using the same restriction enzymes, and the fragment with the correct sized fragment is isolated by gel-purification. Purified PCR product is ligated into the linearized pIVEX 2.3-MCS vector and *E. coli* cells transformed for plasmid amplification. The newly constructed expression vector is verified by restriction mapping and used for protein production.

[0259] *E. coli* lysate is reconstituted with 0.25 ml of Reconstitution Buffer, the Reaction Mix is reconstituted with 0.8 ml of Reconstitution Buffer; the Feeding Mix is reconstituted with 10.5 ml of Reconstitution Buffer; and the Energy Mix is reconstituted with 0.6 ml of Reconstitution Buffer. 0.5 ml of the Energy Mix was added to the Feeding Mix to obtain the Feeding Solution. 0.75 ml of Reaction Mix, 50 µl of Energy Mix, and 10 µg of the template DNA is added to the *E. coli* lysate.

[0260] Using the reaction device (Roche Biochem), 1 ml of the Reaction Solution is loaded into the reaction compartment. The reaction device is turned upside-down and 10 ml of the Feeding Solution is loaded into the feeding compartment. All lids are closed and the reaction device is loaded into the RTS500 instrument. The instrument is run at 30°C for 24 hours with a stir bar speed of 150 rpm. The pIVEX 2.3 MCS vector includes a nucleotide sequence that encodes six consecutive

histidine amino acids on the C-terminal end of the target polypeptide for the purpose of protein purification. Target polypeptide is purified by contacting the contents of reaction device with resin modified with Ni^{2+} ions. Target polypeptide is eluted from the resin with a solution containing free Ni^{2+} ions.

Example 11

Cellular Production of Target Polypeptides

[0261] Nucleic acids are cloned into DNA plasmids having phage recombination cites and target polypeptides are expressed therefrom in a variety of host cells. Alpha phage genomic DNA contains short sequences known as attP sites, and *E. coli* genomic DNA contains unique, short sequences known as attB sites. These regions share homology, allowing for integration of phage DNA into *E. coli* via directional, site-specific recombination using the phage protein Int and the *E. coli* protein IHF. Integration produces two new att sites, L and R, which flank the inserted prophage DNA. Phage excision from *E. coli* genomic DNA can also be accomplished using these two proteins with the addition of a second phage protein, Xis. DNA vectors have been produced where the integration/excision process is modified to allow for the directional integration or excision of a target DNA fragment into a backbone vector in a rapid *in vitro* reaction (Gateway™ Technology (Invitrogen, Inc.)).

[0262] A first step is to transfer the nucleic acid insert into a shuttle vector that contains attL sites surrounding the negative selection gene, ccdB (*e.g.* pENTER vector, Invitrogen, Inc.). This transfer process is accomplished by digesting the nucleic acid from a DNA vector used for sequencing, and to ligate it into the multicloning site of the shuttle vector, which will place it between the two attL sites while removing the negative selection gene ccdB. A second method is to amplify the nucleic acid by the polymerase chain reaction (PCR) with primers containing attB sites. The amplified fragment then is integrated into the shuttle vector using Int and IHF. A third method is to utilize a topoisomerase-mediated process, in which the nucleic acid is amplified via PCR using gene-specific primers with the 5' upstream primer containing an additional CACC sequence (*e.g.*, TOPO® expression kit (Invitrogen, Inc.)). In conjunction with Topoisomerase I, the PCR amplified fragment can be cloned into the shuttle vector via the attL sites in the correct orientation.

[0263] Once the nucleic acid is transferred into the shuttle vector, it can be cloned into an expression vector having attR sites. Several vectors containing attR sites for expression of target polypeptide as a native polypeptide, N-fusion polypeptide, and C-fusion polypeptides are commercially available (*e.g.*, pDEST (Invitrogen, Inc.)), and any vector can be converted into an expression vector for receiving a nucleic acid from the shuttle vector by introducing an insert having an attR site flanked by an antibiotic resistant gene for selection using the standard methods described above. Transfer of the nucleic acid from the shuttle vector is accomplished by directional recombination using Int, IHF, and Xis (LR clonase). Then the desired sequence can be transferred to an expression vector by carrying

out a one hour incubation at room temperature with Int, IHF, and Xis, a ten minute incubation at 37°C with proteinase K, transforming bacteria and allowing expression for one hour, and then plating on selective media. Generally, 90% cloning efficiency is achieved by this method. Examples of expression vectors are pDEST 14 bacterial expression vector with att7 promoter, pDEST 15 bacterial expression vector with a T7 promoter and a N-terminal GST tag, pDEST 17 bacterial vector with a T7 promoter and a N-terminal polyhistidine affinity tag, and pDEST 12.2 mammalian expression vector with a CMV promoter and neo resistance gene. These expression vectors or others like them are transformed or transfected into cells for expression of the target polypeptide or polypeptide variants. These expression vectors are often transfected, for example, into murine-transformed a adipocyte cell line 3T3-L1, (ATCC), human embryonic kidney cell line 293, and rat cardiomyocyte cell line H9C2.

Representative Nucleotide and Amino Acid Sequences

[0261] Following is a genomic nucleotide sequence for a *CTEP* region. The genomic nucleotide sequence is set forth in SEQ ID NO: 1. The following nucleotide representations are used throughout: "A" or "a" is adenosine, adenine, or adenylic acid; "C" or "c" is cytidine, cytosine, or cytidylic acid; "G" or "g" is guanosine, guanine, or guanylic acid; "T" or "t" is thymidine, thymine, or thymidylic acid; and "I" or "i" is inosine, hypoxanthine, or inosinic acid. Exons are indicated in italicized lower case type, introns are depicted in normal text lower case type, and polymorphic sites are depicted in bold upper case type. SNPs are designated by the following convention: "R" represents A or G, "M" represents A or C; "W" represents A or T; "Y" represents C or T; "S" represents C or G; "K" represents G or T; "V" represents A, C or G; "H" represents A, C, or T; "D" represents A, G, or T; "B" represents C, G, or T; and "N" represents A, G, C, or T.

>16:56742951-56841200

```
1       tgtctaaaaa ttattatggc tgaaacatac ttatcatttg ttttctgtag tagaaaatgg
61      catatatatga gaaggcctac tttgtaatag ttgtttgctt tacttaatat ttttcaaatc
121     agccatctgt gtgaagactc aatatcaagt taggcctta aaaggggga aataattcaa
181     aaccactggc agctgtggtt ttccRgcctt ttctattggt tgaactctgt aataaatgca
241     ggaccctaca tatacaaaag gtactgtttt ccaaattcac gtatgtatta gcatttacct
301     ttaaagttct catccgtcag aaaaacggtg aacacagcat tcagtatatt tatttggtt
361     tagttacaca gtgtcaaaaa gttctgatgt atatggataa gtagatgcac tttttgctgt
421     tttgtttgtt taaacagctt gctttaatta tctcaagcta cttggcagga acacctttat
481     tccaggtttc gcataatcag ttattctggc aagagtagat taacacgtta gtgatctcca
541     atagacagat tatatgttaa tattatttga ggccattatt tttaagtaca atttagacgg
601     agtttcaaat agattttaaa aatgagattt gtataaaaga gtgtgcagtc cctatggtgt
661     taaaatcact gatatggagg agaagagggg ttggaattgt gacactgtgg cagcagatga
721     cctttggacaa gtcagttttt cagactttcc tcccttaaaa tagggatact aaatctcaca
781     gggtggttgt gtataatgtc acccacacgc catctcctct ataactgcat ttgtatgcca
841     ggaaattgtc tgtgaaggct tcagtttaga atagcttcat attattttgg ggcactgctg
901     ggtgattttg gttaaggctg gccttgggag ttctctgagt tttctctgaa accaaactca
961     atttggttgt aggttccctg tttgctgttt gttgcttttа gtaatagggag ttagtcagtt
1021    tacctgggag ttaagtttgg actggagata atttgccatt ctttttttt ttttttttt
1081    ttttttttga gatagggtct cactctgtcg cccaggctgg agtgcagtga catgatcttg
1141    gctcactgca acctctgcct ccctggttca agcgattccc ctgcctcagc ctcctgagta
1201    gttgggtta caggcacaag ccaccacgcc cagctaattt ttgcattttt agtagagatg
1261    gggttcacc atgttggcca ggctggtctc gaaccctga cctcaggtga tctgcccgcc
1321    ttggcctccc acagtgcttg gattacaggc atgagccacc atgcccagcc gataatttgc
1381    cattctgtat ttatttgcgt gttagagacc agttaaagtt aagacacttt ctaaatagaa
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1441 gtcttgtata tggggctttt gagaaagtgt tttcccgtga tgctgggggg aaacacacat
1501 attcaaatta cttgtataaa acagtttatt ctgtgtttag gaacttgtag ggtgaaatg
1561 ctgacctgtg ttactctttc tttccatgat cagcRtccag aggaccagag gttaatttat
1621 tctgggaagc tgttgttgga tcaccaatgt ctcagggact tgcttccaaa ggtacatcac
1681 ttacacatta acttctgaat gtttttaagc actcaccagg ttaggttcag gtccttaagt
1741 accttcgtat tacttttagc aggaaaaacg gcatgttttg catctggtgt gcaatgtgaa
1801 gagtccttca aaaatgccag aaatcaacgc caaggtgtgt ctgcctcttc atgatggtaa
1861 caatttgtat cattcagact ttcaggggag taataaaaga agttggataa aacgtttatt
1921 gagagactgt atggtgttat taccaagtaa tattttgttt ctttcctagg aattagaaac
1981 tgtattatca acacaaagtg atgagttaat agtagcagat tgttccgtta gtgctgtagt
2041 agaaaggtgg agttacatat tgcactatct tatgggatat gatttgatct atggttactg
2101 tgtatctaga ttactgaact aacactaatc gggtttttat tgaatttagc acatagcaag
2161 tttcttcaaa ggagtcacta atttttataa agagtacaaa agtgaatata tttcctttgg
2221 aaattttcat tgtgattagt tggataggaa aagatcagag ttttttatcaa gtgatcttta
2281 aagaattttt ttttttaaa aatggtctcg ctgtgtggcc caggcttttc tcaaactcct
2341 gagggcaagc gatcctccca cctcagcctc ctgagtagct gggactacag gcatgtgcca
2401 ctagacctgg ctctaaagac atatatgaca cacgaaacca tttatttttc atttcacaat
2461 gtttattcac atatatggta ttagtattct aatgtagtga tgcactctaa atttgcatta
2521 tatttcctag aacatctgaa cagagcatag gaaattccct attttgccat tatcagttct
2581 aacaaaaatc ttaaaagcac tttatcattt catttccctg cactgtaatt tttttaaatg
2641 atcaaaaacR gtatcatacc aaggcttact tatattggaa tactatttta gaaagttgtg
2701 ggctgggttg tatttataaa tcttgttggt cagatgtctg caatgagtaa atttagcacc
2761 attatcagga agctttctca ccaatgacaa cttcattgga agattttaat gaaagtgtag
2821 catactctag ggaaaaaata tgaatatttt agcatctatg tattgaaaat tatgttgaat
2881 aaatgtcaga ctattttttta cataacgttg cttctgttta attttgtcac gttcagaggt
2941 ggggggtagg agatgtaagc ccttgacagc aaaataattc cttttgcttg atttcagaca
3001 gttgcatcag ctcctttgtt ctgtgttcat gttacactta tttaggtggc tgaatccaca
3061 gaggagcctg ctggttctaa tcggggacag tatcctgagg attcctcaag tgatggttta
3121 aggcaaaggg aagttcttcg gaacctttct tcccctggat gggaaaacat ctcaaggtga
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97321  tggtggtgca tgcctgtggt tccagctatt tgggaggctg aggtgggaag attgcttgag
97381  ccgtggaggt tgaggctgca gtgaggtgtg aacacaccac tgcactccag cctgagtagc
97441  agagtgagat ctccatctca aatttaaaaa gaaaggaaaa aaaaaaactc acaacattct
97501  taattttttat tatatttctt gtgaaaattg tttttctgac ttttaaaac actcttcgag
97561  gattcatcaa gcaaagacat aatcagtatt tcacatgcct gatttaaaac actttgcta
97621  gttctcgtat gtttccctgt caattgcaaa atcagcttta tctggatgtt tgaacattg
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97921  ggtgatggga attcttcctg ctactgctgc aatgaggaat gaattactgc ttaggttggc
97981  aaaggatttt aacttcctct agYgtgtcca gaccacatct ggtccctgtg gaaggaagca
98041  tccccagaag actcagagga aggcaaaccc ttggggagaa cataaagata ctgttagact
98101  ggcattacta gatggtgaaa actctggaaa agcccttaca actgaaaaaa gacagggagg
98161  gcaattatgg tacctacatc ttagggttgt tctgagaatt aaatgagtta ctacacttaa
98221  ggagtttaga gcactgttgg catgcagtgg
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Following is a genomic nucleotide sequence of a *PROL4* region (SEQ ID NO: 2).

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181    aaaggctttg gtgaccctga gctctgctca gagcatagat gatggcaaRa ggaggaaatg
241    gggacaatac aatggataac ggggttaaaa tgcatggaaa gtactagagt tagattgacc
301    ctttcagaaa ttaagcactt tattttcaga gagtaggtca ataaaagagg aggaaacctt
361    aattttctRtt tttcactgat tgcagatact tctctgaaaa tctcggcctа agtcttaagt
421    caacgtcttc atgatgtaat tttgccgttc taatgtatat ggatttтcag ttgttgattc
481    tgtttgtgtg aatcagggca tctaaattga acggaaaatc tatatttttc atttctataa
541    tctataatag aattctataa ttctataatt catctagata tcRcatttcc ttgacatcaa
601    atctcttgaac attaaaaaat attgataatt ttgattaaat ttgtacttaa tcaaaatttt
661    gatgctaagc aggcactatt aaattctttc tattccattc cttaatcctt taaaatacag
721    gtcacccctc attcagagtc aatgattggg actaggggttc tacttтggga aactttggtt
781    tattgtatcc aaatctttat aatttttttt ccctaaagct ctcttaggca tttttaaatc
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841   tcttgtgtct aaacctaagt gtctttctag aaattcacag ctggggtttt atcaaatcat
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1741  tgccactctg aattaaaaca caaatgcttc attctttagt tttcaagtcc tgataactgc
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2401  cctaaaatat ctttgccaca ataYgaatga tgtgcttttg atggtatgtc ccagatacat
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Following is a genomic nucleotide sequence of a *GRID2* region (SEQ ID NO: 3).

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Following is a genomic nucleotide sequence of a *PDE4D* region (SEQ ID NO: 4).

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Following is a genomic nucleotide sequence of a *GPX3* region (SEQ ID NO: 5).

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92161 ccgcttgccc aaaccagaaa cagctggtgg tatagaagca gccagcgcag actttcttcc
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96121 tactgaagaa tgtcctttct caatgacaac aacctgcatg tatgcctaac tggtgagaac
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96301 tggccaacat ggcaaaaccc agtctctact aaaaatacaa aaattagccg ggtgtggtgg
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96601  ttgcatcagt acacagaagc tggacaaaaa tatgcactgc cacatgcttt gtaatagaca
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96721  Ratggaatac tatacagcca tcaaaaagaa tggggtagat tgcttggcaa tgatacagaa
96781  agatgcccat aaaatacaaa gcaaagtgca gatttatatg tgtaacagga tgctatttttt
96841  ttcaaaaaca cattgtgtgt gtgtgtgtat acatacggaa tcttaaatgt gcatagaaaa
96901  aggcctggaa gcccagtgct ttgggaggcc aaggtgggag gattgcttga ggccaggggt
96961  tcaagaccag cctgggcaaa aagcaagaat agatttctcta ttaaaaaaaa acttttttac
97021  attagcagag catggtggca catgcctgga gtcccagcta ctcggaaagt tgaggtggga
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97141  gcctgagtga cagactaata ctctgtctct taaaaaaaag aaaagaagaa aagaaaaaag
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97561  gtagtcccag ctactccaga gacagacgca ggaggatggc ctgaaaccag agtttatcta
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97681  caaaatatgg tggaaagagg gtttcacaga ttcaaatcct ggctatgatc ccttctcgaa
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97981  atcaatacat tgtggcaatt tcctatcagg aaatactata cagcagtgaa aaaagaatga
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98401  cattaaaaaa aaaactttaa agaaatgcaa gaaaaaaacc ccactaaatt cagaacactt
98461  gtttcttgtt ggtggctgat gaaggagaaa tttcaaStga ttggaaatgt gttatttctt
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98581  tcatttttcc tttttcttt ttactgtatt gtttgctttt tttctgctca aactttatt
98641  ttagattcgg ggggtacatg tgcaggtttg ctacctgggt atactgcgtg atttgggtag
98701  gggtgatccc atcacctggg tactgagcat agttcccaac agttttttcaa ctctgcctcc
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98941  tcacgcagga gttgcacctg cagagacaga agtgggctgg agagggacca gggctcctaa
99001  gtctgctgct gaagctgact atagcgaagg caaaggacaa gtggcctgaa ggccaacaga
99061  gagcctggcc aatgctctgc ccagtgggtg gagactgagc caaaggccct tgactcatag
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99421  gaaaatcatc aagaacttcc cRcaatggtc atggtggccg ccaaggtctt tctctggggg
99481  ttgggagctg tgctccccca caaaacaagg ggacccagct gccctgtgcc ttcctgtggc
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99601  cactgcaggc atgtgggggc atagcagcaa gaatctagga gggcaggcac agcaaggcct
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99721  ttatagagtt tttaagactt ggaagagaat gccctgagat gccaNagagt gaaacatttt
99781  cattctaaag ctgggaaacc aaagtcggca aaggcgaaca gacgtgccca gagccacatg
99841  ggacagcagt ggcacagaag aaatgcacac ctcgtcctct ggtggccact gtgcactctc
99901  ttaccacacc atgctactgc tcaacctctc cccctccctc acagtggtca ggtaagcaat
99961  gagggaaaat gatggaggtg agaaaggaag aacccgggct
```

Following is a *CETP* coding nucleotide sequence (cDNA, SEQ ID NO: 6).

>gi|4557442|ref|NM_000078.1| Homo sapiens cholesteryl ester transfer protein, plasma (CETP), mRNA.

```
  1 gtgaatctct ggggccagga agaccctgct gcccggaaga gcctcatgtt ccgtgggggc
 61 tgggcggaca tacatatacg ggctccaggc tgaacggctc gggccactta cacaccactg
```

```
 121 cctgataacc atgctggctg ccacagtcct gaccctggcc ctgctgggca atgcccatgc
 181 ctgctccaaa ggcacctcgc acgaggcagg catcgtgtgc cgcatcacca agcctgccct
 241 cctggttgtg aaccacgaga ctgccaaggt gatccagacc gccttccagc gagccagcta
 301 cccagatatc acgggcgaga aggccatgat gctccttggc caagtcaagt atgggttgca
 361 caacatccag atcagccact tgtccatcgc cagcagccag gtggagctgg tggaagccaa
 421 gtccattgat gtctccattc agaacgtgtc tgtggtcttc aaggggaccc tgaagtatgg
 481 ctacaccact gcctggtggc tgggtattga tcagtccatt gacttcgaga tcgactctgc
 541 cattgacctc cagatcaaca cacagctgac ctgtgactct ggtagagtgc ggaccgatgc
 601 ccctgactgc tacctgtctt tccataagct gctcctgcat ctccaagggg agcgagagcc
 661 tgggtggatc aagcagctgt tcacaaattt catctccttc accctgaagc tggtcctgaa
 721 gggacagatc tgcaaagaga tcaacgtcat ctctaacatc atggccgatt ttgtccagac
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1021 tggccgcctc atgctcagcc tgatgggaga cgagttcaag gcagtgctgg agacctgggg
1081 cttcaacacc aaccaggaaa tcttccaaga ggttgtcggc ggcttcccca gccaggccca
1141 agtcaccgtc cactgcctca agatgcccaa gatctcctgc caaaacaagg gagtcgtggt
1201 caattcttca gtgatggtga aattcctctt tccacgccca gaccagcaac atttctgtagc
1261 ttacacattt gaagaggata tcgtgactac cgtccaggcc tcctattcta agaaaaagct
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1381 cagctccgag tccatccaga gcttcctgca gtcaatgatc accgctgtgg gcatccctga
1441 ggtcatgtct cggctcgagg tagtgtttac agcctcatg aacagcaaag gcgtgagcct
1501 cttcgacatc atcaaccctg agattatcac tcgagatggc ttcctgctgc tgcagatgga
1561 cttggcttc cctgaggcac tgctggtgga tttcctccag agcttgagct agaagtctcc
1621 aaggaggtcg ggatgggcct tgtagcagaa ggcaagcacc aggctcacag ctggaacctt
1681 ggtgtctcct ccagcgtggt ggaagttggg ttaggagtac ggagatggag attggctccc
1741 aactcctccc tatcctaaag gcccactggc attaaagtgc tgtatccaag
```

CDS: 131-1612 base pairs

Signal peptide: 131-181 base pairs

Mature peptide: 182-1609 base pairs

PolyA signal: 1771-1776 base pairs

Following is a second *PROL4* cDNA sequence (SEQ ID NO:7). >gi|6005801|ref|NM_007244.1| Homo sapiens proline rich 4 (lacrimal) (PRR4), mRNA

```
   1 cagagcctcc ttcaagatgc tgctggtcct gctctcagtg gtccttctgg ctctgagctc
  61 agctcagagc acagataatg atgtgaacta tgaagacttt actttcacca taccagatgt
 121 agaggactca agtcagagac cagatcaggg acccccagaga cctcctcctg aaggactcct
 181 acctagaccc cctggtgata gtggtaacca agatgatggt cctcagcaga gaccaccaaa
 241 accaggaggc catcaccgcc atcctccccc acctcctttt caaaatcagc aacgaccacc
 301 ccaacgagga caccgtcaac tctctctacc ccgatttcct tctgtcagcc tgcaggaagc
 361 atcatcattc ttccggaggg acagaccagc aagacatccc caggagcaac cactctggta
 421 atctagaatt cagtggcaga aaataaataa gaagataact tccttcagaa agccatgaca
 481 ttgaaataat gtggtcataa ctctttc
```

CDS: 17..421 base pairs

PolyA signal: 442..447 base pairs

Following is a *GRID2* cDNA sequence (SEQ ID NO: 8). >gi|4557632|ref|NM_001510.1| Homo sapiens glutamate receptor, ionotropic, delta 2 (GRID2), mRNA.

```
   1 atggaagttt tccccttgct cttggttttg tccgtctggt ggtctcgaac ctgggactcg
  61 gcgaatgcgg attcgatcat tcacatcgga gcaatttttg atgaatctgc caaaaaggat
```

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 121 gatgaggtat ttcgcactgc ggttggtgac cttaaccaga atgaagagat cttacagact
 181 gagaaaatca cattttcagt gacgtttgtt gatggcaaca acccttttca agcagttcaa
 241 gaagcctgtg aacttatgaa tcaaggcatc ttggccctgg tcagctccat tggctgcacg
 301 tcagcaggat ccctccagtc tttggcagac gccatgcata tcccccacct cttcattcag
 361 cgctcaacag ctgggacccc aaggagtggc tgtggactca cccggagcaa caggaatgat
 421 gactacactc tctcagttcg cccacctgtc tacttgcatg atgttatcct aagagtggtc
 481 acagagtatg cctggcagaa attcattata ttctatgata gtgaatacga tatccgtgga
 541 atacaggagt tcttggacaa agtctctcag cagggaatgg atgttgcact tcagaaggta
 601 gaaaacaaca tcaataaaat gattaccact ctctttgaca ccatgagaat agaagaactg
 661 aatcgctatc gagacactct taggcgagcg atccttgtta tgaatcctgc tacagccaaa
 721 tccttcatta ctgaggttgt ggagactaat ttggttgctt ttgactgtca ctggatcatt
 781 atcaaatgag aaataaacga tgtggacgta caggaacttg taagaaggtc aattggaagg
 841 ttaacgatta ttcgggcagc atttccaatt cccagaaaca taagtcaggg gtgttccgct
 901 ggcaaccatc gaatatcttc aacattgtgt gatccaaagg atccatttgc tcagaatatg
 961 gagatttcca acctttacat atatgacacg gtgcttctgc ttgctaatgc ttttcataag
1021 aagctggagg accgaaagtg gcacagcatg gcaagtctgt catgtatcag aaagaactca
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1501 gaagatggga catggaatgg cttggtagga gaacttgtct ttaagagagc cgacataggg
1561 atttctgctt taaccatcac tccagatcgt gaaaatgtgg tggactttac gacacgttac
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2461 cagaaaggag gcgccctgga cataaagagc tttgcagggg tcttttgtat cctggctgct
2521 ggaatgtgtc tctcctgctt catagccatg ctggagacgt ggtggaacaa gaggaaaggc
2581 tcccgggttc catcaaaaga ggatgacaag gaaattgacc tggagcacct ccatagacgt
2641 gtaaatagct tgtgcacaga tgacgacagc ccccataaac agttttccac ctcgtcaatt
2701 gatttgaccc ctctggacat tgacactttg ccaacacgac aagcactgga gcaaatcagt
2761 gatttcagga acactcatat taccacaaca accttatcc cagagcagat ccagactctt
2821 agccgcacac tgtcagctaa agctgcttct ggtttcactt ttggcaacgt gcctgagcac
2881 cgaactggcc cttttaggca cagggcacct aatgggggct ttttcaggag tcctataaaa
2941 acaatgtcat ctattcctta tcaaccaact cctaccctgg ggctcaatct gggtaatgat
3001 ccagaccgag gcacctccat atga
```

Following is a *PDE4D* cDNA sequence (SEQ ID NO: 9). >gi|46361981|ref|NM_006203.3| Homo sapiens phosphodiesterase 4D, cAMP-specific (phosphodiesterase E3 dunce homolog, Drosophila) (PDE4D), mRNA.

```
   1 ggaattcatc tgtaaaaatc actacatgta acgtaggaga caagaaaaat attaatgaca
  61 gaagatctgc gaacatgatg cacgtgaata attttccctt tagaaggcat tcctggatat
 121 gttttgatgt ggacaatggc acatctgcgg gacggagtcc cttggatccc atgaccagcc
 181 caggatccgg gctaattctc caagcaaatt ttgtccacag tcaacgacgg gagtccttcc
 241 tgtatcgatc cgacagcgat tatgacctct ctccaaagtc tatgtcccgg aactcctcca
 301 ttgccagtga tatacacgga gatgacttga ttgtgactcc atttgctcag gtcttggcca
 361 gtctgcgaac tgtacgaaac aactttgctg cattaactaa tttgcaagat cgagcaccta
 421 gcaaaagatc acccatgtgc aaccaaccat ccatcaacaa agccaccata acagaggagg
 481 cctaccagaa actggccagc gagaccctgg aggagctgga ctggtgtctg gaccagctag
 541 agacctcaca gaccaggcac tccgtcagtg agatggcctc caacaagttt aaaaggatgc
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601 ttaatcggga gctcacccat ctctctgaaa tgagtcggtc tggaaatcaa gtgtcagagt
661 ttatatcaaa cacattctta gataagcaac atgaagtgga aattccttct ccaactcaga
721 aggaaaagga gaaaaagaaa agaccaatgt ctcagatcag tggagtcaag aaattgatgc
781 acagctctag tctgactaat tcaagtatcc caaggtttgg agttaaaact gaacaagaag
841 atgtccttgc caaggaacta gaagatgtga acaaatgggg tcttcatgtt ttcagaatag
901 cagagttgtc tggtaaccgg cccttgactg ttatcatgca caccatttct cggagaacggg
961 atttattaaa aacatttaaa attccagtag atactttaat tacatatctt atgactctcg
1021 aagaccatta ccatgctgat gtggcctatc acaacaatat ccatgctgca gatgttgtcc
1081 agtctactca tgtgctatta tctacacctg ctttggaggc tgtgtttaca gatttggaga
1141 ttccttgcagc aatttttgcc agtgcaatac atgatgtaga tcatcctggt gtgtccaatc
1201 aatttctgat caatacaaag tctgaacttg ccttgatgta caatgattcc tcagtcttag
1261 agaaccatca tttggctgtg ggctttaaat tgcttcagga agaaactgt gacatttcc
1321 agaatttgac caaaaaacaa agacaatctt taaggaaaat ggtcattgac atcgtacttg
1381 caacagatat gtcaaaacac atgaatctac tggctgattt gaagactatg gttgaaacta
1441 agaaagtgac aagctctgga gttcttcttc ttgataatta ttccgatagg attcaggttc
1501 ttcagaatat ggtgcactgt gcagatctga gcaacccaac aaagcctctc cagctgtacc
1561 gccagtggac ggaccggata atggaggagt tcttccgcca aggagaccga gagaggggaac
1621 gtggcatgga gataagcccc atgtgtgaca agcacaatgc ttccgtggaa aaatcacagg
1681 tgggcttcat agactatatt gttcatcccc tctgggagac atgggcagac ctcgtccacc
1741 ctgacgccca ggatattttg gacactttgg aggacaatcg tgaatggtac cagagcacaa
1801 tccctcagag cccctctcct gcacctgatg acccagagga gggccggcag ggtcaaactg
1861 agaaattcca gtttgaacta actttagagg aagatggtga gtcagacacg gaaaaggaca
1921 gtggcagtca agtggaagaa gacactagct gcagtgactc caagactctt tgtactcaag
1981 actcagagtc tactgaaatt ccccttgatg aacaggttga agaggaggca gtaggggaag
2041 aagaggaaag ccagcctgaa gcctgtgtca tagatgatcg ttcctcctgac acgtaacagt
2101 gcaaaactt tcatggcttt tttatttca agtagaaaaa ttgttccaa agtgcatgtc
2161 acatgccaca accacaggtca cacctcactg tcatctgcca ggacgttgt tgaacaaaac
2221 tgaccttgac tactcagtcc agcgctcagg aatatcgtaa ccagttttct cacctccatg
2281 tcatccgagc aaggtggaca tcttcacgaa cagcgttttt aacaagattt cagcttggta
2341 gagctgacaa agcagataaa atctactcca aattattttc aagagagtgt gactcatcag
2401 gcagcccaaa agtttattgg acttggggtt tctattcctt tttatttgtt tgcaatattt
2461 tcagaagaaa ggcattgcac agagtgaact taatggacga agcaacaaat atgtcaagaa
2521 caggacatag cacgaatctg ttaccagtag gaggaggatg agccacagaa attgcataat
2581 tttctaattt caagtcttcc tgatacatga ctgaatagtg tggttcagtg agctgcactg
2641 acctctacat tttgtatgat atgtaaaaca gatttttttgt agagcttact tttattatta
2701 aatgtattga ggtattatat ttaaaaaaaa ctatgttcag aacttcatct gccactggtt
2761 atttttttct aaggagtaac ttgcaagttt tcagtacaaa tctgtgctac actggataaa
2821 aatctaattt atgaatttta cttgcacctt atagttcata gcaattaact gatttgtagt
2881 gattcattgt ttgttttata taccaatgac ttccatattt taaaagagaa aaacaacttt
2941 atgttgcagg aaacccttct tgtaagtctt tattatttac tttgcatttt gtttcactct
3001 ttctcagata gcagagttgc tcttcaccag tgttttctct catgtgcaaa gtgactattt
3061 gttctataat actttttatgt gtgttatatc aaatgtgtct taagcttcat gcaaactcag
3121 tcatcagttc gtgttgtctg aagcaagtgg gaaatatata aactcacagt agctaaaatg
3181 gtcagtcttt tttagatgtt ttcctactta gtatctccta ataacgtttt gctgtgtcac
3241 tagatgttca tttcacaagt gcatgtcttt ctaataatcc acacatttca tgctctaata
3301 atccacacat ttcatgctca tttttattgt ttttacagcc agttatagca agaaaaaggt
3361 ttttcccctt gtgctgcttt ataatttagc gtgtgtctga accttatcca tgtttgctag
3421 atgaggtctt gtcaaatata tcactaccat tgtcaccggt gaaaagaaac aggtagttaa
3481 gttagggtta acattcattt caaccacgag gttgtatatc atgactagct tttactcttg
3541 gtttacagag aaaagttaaa caaccaacta ggcagttttt aagaatatta acaatatatt
3601 aacaaacacc aatacaacta atcctatttg gttttaatga ttttaccatg ggattaagaa
3661 ctatatcagg aacatccctg agaaacggct ttaagtgtag caactactct tccttaatgg
3721 acagccacat aacgtgtagg aagtccttta tcacttatcc tcgatccata agcatatctt
3781 gcagagggga actactttct taaacacatg gagggaaaga agatgatgcc actggcacca
3841 gatgggttagt actgtgatgc atcctaaaat atttattata ttggttaaaa ttttggttaa
3901 atcaaaaatt agagatcact cttggctgat ttcagcacca gggactgtat tacagctttg
3961 gagattaatt cctagtgttt acctgattat agcagttggc atcatggggc atttaattct
4021 gactttatcc ccacgtcagc cttaataaag tcttctttac cttctctatg aagactttaa
4081 agcccaaata atcatttttc acattgatat tcaagaattg agatagatag aagccaaagt
4141 gggtatctga caagtggaaa atcaaacgtt taagaagaat tacaactctg aaaagcattt
4201 atatgtggaa cttctcaagg agcctcctgg ggactggaaa gtaagtcatc agccaggcaa
4261 atgactcatg ctgaagagag tccccatttc agtccccctga gatctagctg atgcttagat
4321 cctttgaaat aaaaattatg tctttataac tctgatcttt tacataaagc agaagaggaa
4381 tcaactagtt aattgcaagg tttctactct gtttcctctg taaagatcag atggtaatct
4441 ttcaaataag aaaaaaataa agacgtatgt ttgaccaagt agtttcacaa gaatatttgg
4501 gaacttgttt cttttaattt tatttgtccc tgagtgaagt ctagaaagaa aaggtaaagag
4561 tctagagttt attcctcttt ccaaaacatt ctcattcctc tcctccctac acttagtatt
4621 tcccccacag agtgcctaga atcttaataa tgaataaaat aaaaagcagc aatatgtcat

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4681 taacaaatcc agacctgaaa gggtaaaggg tttataactg cactaataaa gagaggctct
4741 ttttttttct tccagtttgt tggtttttaa tggtaccgtg ttgtaaagat acccactaat
4801 ggacaatcaa attgcagaaa aggctcaata tccaagagac agggactaat gcactgtaca
4861 atctgcttat ccttgccctt ctctcttgcc aaagtgtgct tacagaatat atactgcttt
4921 aaaaaagaat aaaagaatat ccttttacaa gtggctttac atttcctaaa atgccataag
4981 aaaatgcaat atctgggtac tgtatgggga aaaaaatgtc caagtttgtg taaaaccagt
5041 gcatttcagc ttgcaagtta ctgaacacaa taatgctgtt ttaattttgt tttatatcag
5101 ttaaaattca caataatgta gatagaacaa attacagaca aggaaagaaa aaacttgaat
5161 gaaatggatt ttacagaaag ctttatgata atttttgaat gcattattta ttttttgtgc
5221 catgcatttt ttttctcacc aaatgacctt acctgtaata cagtcttgtt tgtctgttta
5281 caaccatgta tttattgcaa tgtacatact gtaatgttaa ttgtaaatta tctgttctta
5341 ttaaaacatc atcccatgat ggggtggtgt tgatatattt ggaaactctt ggtgagagaa
5401 tgaatggtgt gtatacatac tctgtacatt tttcttttct cctgtaatat agtcttgtca
5461 ccttagagct tgtttatgga agattcaaga aaactataaa atacttaaag atatataaat
5521 ttaaaaaaac atagctgcag gtcttggtcc ccagggctgt gccttaactt taaccaatat
5581 tttcttctgt tttgctgcat ttgaaaggta acagtggagc taggggtggg cattttacat
5641 ccaggctttt aattgattag aattctgcca atagggtggt tttacaaaac cacagacaac
5701 ctctgaaaga ttctgagacc cttttgagac agaagctctt aagtacttct tgccagggag
5761 cagcactgca tgtgtgatgg ttgtttgcca tctgttgatc aggaactact tcagctactt
5821 gcatttgatt atttcctttt ttttttttt taactcggaa acacaactgg gggaat
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Following is a *GPX3* cDNA sequence (SEQ ID NO: 10). >gi|6006000|ref|NM_002084.2| Homo sapiens glutathione peroxidase 3 (plasma) (GPX3), mRNA.

```
   1 agccaaaaga ggaagggacc ggcctcccac gtccacaggg acctgacttc cacctctctg
  61 cccagatttg cttatgtcac tgtcgccccg ggacggggag gtggggagct gagggcaagt
 121 cgcgcccgcc cctgaaatcc cagccgccta gcgattggct gcaagggtct cggcttggcc
 181 gcggattaat cacacccgag ggcttgaaag gtggctggga gcgccggaca cctcagacgg
 241 acggtggcca gggatcaggc agcggctcag gcgaccctga gtgtgccccc accccgccat
 301 ggcccggctg ctgcaggcgt cctgcctgct ttccctgctc ctggccggct tcgtctcgca
 361 gagccgggga caagagaagt cgaagatgga ctgccatggt ggcataagtg gcaccattta
 421 cgagtacgga gccctcacca ttgatgggga ggagtacatc cccttcaagc agtatgctgg
 481 caaatacgtc ctctttgtca acgtggccag ctactgaggc ctgacgggcc agtacattga
 541 actgaatgca ctacaggaag agcttgcacc attcggttctg gtcattctgg gctttccctg
 601 caaccaattt ggaaaacagg aaccaggaga gaactcagag atccttcctа ccctcaagta
 661 tgtccgacca ggtggaggct ttgtccctaa tttcagctc tttgagaaag gggatgtcaa
 721 tggagagaaa gagcagaaat tctacacttt cctaaagaac tcctgtcctc ccacctcgga
 781 gctcctgggt acatctgacc gcctctttcg ggaacccatg aaggtcacg acatccgctg
 841 gaacttgag aagttcctgg tggggccaga tggtatаccc atcatgcgct ggcaccaccg
 901 gaccacggtc agcaacgtca agatggacat cctgtcctac atgaggcggc aggcagccct
 961 gggggtcaag aggaagtaac tgaaggccgt ctcatcccat gtccacacatg taggggaggg
1021 actttgttca ggaagaaatc cgtgtctcca accacactat ctacccatca cagacccctt
1081 tcctatcact caaggcccca gcctggcaca aatggatgca tacagttctg tgtactgcca
1141 ggcatgtggg tgtgggtgca atgtgggtgt ttacacacat gcctacaggt atgcgtgatt
1201 gtgtgtgtgt gcatgggtgt acagccacgt gtctacctat gtgtctttct gggaatgtgt
1261 accatctgtg tgcctgcagc tgtgtagtgc tggacagtga caaccctttc tctccagttc
1321 tccactccaa tgataatagt tcacttatac ctaaacccaa aggaaaaacc agctctaggt
1381 ccaattgttc tgctctaact gatacctcaa ccttggggcc agcatctccc actgcctcca
1441 aatattagta actatgactg acgtccccag aagttttctgg gtctaccaca ctccccaacc
1501 ccccactcct acttcctgaa gggccctccc aaggctacat ccccacccca cagttctccc
1561 tgagagagat caacctccct gagatcaacc aaggcagatg tgacagcaag ggccacggac
1621 cccatggcag gggtggcgtc ttcatgaggg aggggcccaa agccctgtg ggcggacctc
1681 ccctgagcct gtctgaggag ccagccctta gtgcattcag gctaaggccc ctgggcaggg
1741 atgccacccc tgctcctctg gaggacgtgc cctcaccccct cactggtcca ctggctgag
1801 actcaccccg tctgcccagt aaaagccttt ctgcagcaaa aaaaaaaaaa aaaaaa
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CDS: 299-979 base pairs

Signal peptide: 299-358 base pairs

Mature peptide: 359-976 base pairs

PolyA signal: 1838 base pairs

Following is a *CETP* amino acid sequence (SEQ ID NO: 11).

>gi|4557443|ref|NP_000069.1| cholesteryl ester transfer protein, plasma precursor [Homo sapiens]

MLAATVLTLALLGNAHACSKGTSHEAGIVCRITKPALLVLNHETAKVIQTAFQRASYPDITGEK
AMMLLGQVKYGLHNIQISHLSIASSQVELVEAKSIDVSIQNVSVVFKGTLKYGYTTAWWLGID
QSIDFEIDSAIDLQINTQLTCDSGRVRTDAPDCYLSFHKLLLHLQGEREPGWIKQLFTNFISFTLK
LVLKGQICKEINVISNIMADFVQTRAASILSDGDIGVDISLLTGDPVITASYLESHHKGHFIYKNVS
EDLPLPTFSPTLLGDSRMLYFWFSERVFHSLAKVAFQDGRLMLSLMGDEFKAVLETWGFNTN
QEIFQEVVGGFPSQAQVTVHCLKMPKISCQNKGVVVNSSVMVKFLFPRPDQQHSVAYTFEEDI
VTTVQASYSKKKKLFLSLLDFQITPKTVSNLTESSSESIQSFLQSMITAVGIPEVMSRLEVVFTAL
MNSKGVSLFDIINPEIITRDGFLLLQMDFGFPEHLLVDFLQSLS

Protein: 1..493 amino acids

Signal peptide: 1-17 amino acids

Mature peptide: 18-493 amino acids

Following is a second *PROL4* amino acid sequence (SEQ ID NO: 12).

>gi|6005802|ref|NP_009175.1| proline rich 4 (lacrimal) [Homo sapiens].

MLLVLLSVVLLALSSAQSTDNDVNYEDFTFTIPDVEDSSQRPDQGPQRPPPEGLLPRPPGDSGN
QDDGPQQRPPKPGGHHRHPPPPPFQNQQRPPQRGHRQLSLPRFPSVSLQEASSFFRRDRPARHP
QEQPLW

Protein: 1..134 amino acids

Signal peptide: 1-16 amino acids

Mature peptide: 17-134 amino acids

Following is a *GRID2* amino acid sequence (SEQ ID NO: 13). >gi|4557633|ref|NP_001501.1| glutamate receptor, ionotropic, delta 2 [Homo sapiens].

MEVFPLLLVLSVWWSRTWDSANADSIIHIGAIFDESAKKDDEVFRTAVGDLNQNEEILQTEKIT
FSVTFVDGNNPFQAVQEACELMNQGILALVSSIGCTSAGSLQSLADAMHIPHLFIQRSTAGTPR
SGCGLTRSNRNDDYTLSVRPPVYLHDVILRVVTEYAWQKFIIFYDSEYDIRGIQEFLDKVSQQG
MDVALQKVENNINKMITTLFDTMRIEELNRYRDTLRRAILVMNPATAKSFITEVVETNLVAFD
CHWIIINEEINDVDVQELVRRSIGRLTIIRQTFPIPQNISQRCFRGNHRISSTLCDPKDPFAQNMEIS
NLYIYDTVLLLANAFHKKLEDRKWHSMASLSCIRKNSKPWQGGRSMLETIKKGGVSGLTGEL
EFGENGGNPNVHFEILGTNYGEELGRGVRKLGCWNPVTGLNGSLTDKKLENNMRGVVLRVV
TVLEEPFVMVSENVLCKPKKYQGFSIDVLDALSNYLGFNYEIYV
APDHKYGSPQEDGTWNGLVGELVFKRADIGISALTITPDRENVVDFTTRYMDYSVGVLLRRAE
KTVDMFACLAPFDLSLWACIAGTVLLVGLLVYLLNWLNPPRLQMGSMTSTTLYNSMWFVYG
SFVQQGGEVPYTTLATRMMMGAWWLFALIVISSYTANLAAFLTITRIESSIQSLQDLSKQTEIPY
GTVLDSAVYEHVRMKGLNPFERDSMYSQMWRMINRSNGSENNVLESQAGIQKVKYGNYAFV
WDAAVLEYVAIYDPDCSFYTIGNTVADRGYGIALQHGSPYRDVFSQRILELQQNGDMDILKHK
WWPKNGQCDLYSSVDTKQKGGALDIKSFAGVFCILAAGIVLSCFIAMLETWWNKRKGSRVPS

KEDDKEIDLEHLHRRVNSLCTDDDSPHKQFSTSSIDLTPLDIDTLPTRQALEQISDFRNTHITTTT
FIPEQIQTLSRTLSAKAASGFTFGNVPEHRTGPFRHRAPNGGFFRSPIKTMSSIPYQPTPTLGLNL
GNDPDRGTSI

Following is a *PDE4D* amino acid sequence (SEQ ID NO: 14). >gi|32306513|ref|NP_006194.2| cAMP-specific phosphodiesterase 4D [Homo sapiens]

MMHVNNFPFRRHSWICFDVDNGTSAGRSPLDPMTSPGSGLILQANFVHSQRRESFLYRSDSDY
DLSPKSMSRNSSIASDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSIN
KATITEEAYQKLASETLEELDWCLDQLETLQTRHSVSEMASNKFKRMLNRELTHLSEMSRSGN
QVSEFISNTFLDKQHEVEIPSPTQKEKEKKKRPMSQISGVKKLMHSSSLTNSSIPRFGVKTEQED
VLAKELEDVNKWGLHVFRIAELSGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDHYH
ADVAYHNNIHAADVVQSTHVLLSTPALEAVFTDLEILAAIFASAIHDVDHPGVSNQFLINTNSE
LALMYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKMVIDIVLATDMSKHMNLL
ADLKTMVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIMEEFFR
QGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEDNRE
WYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCT
QDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT

Following is a *GPX3* amino acid sequence (SEQ ID NO: 15). >gi|6006001|ref|NP_002075.2| plasma glutathione peroxidase 3 precursor [Homo sapiens]

MARLLQASCLLSLLLAGFVSQSRGQEKSKMDCHGGISGTIYEYGALTIDGEEYIPFKQYAGKY
VLFVNVASYUGLTGQYIELNALQEELAPFGLVILGFPCNQFGKQEPGENSEILPTLKYVRPGGG
FVPNFQLFEKGDVNGEKEQKFYTFLKNSCPPTSELLGTSDRLFWEPMKVHDIRWNFEKFLVGP
DGIPIMRWHHRTTVSNVKMDILSYMRRQAALGVKRK

[0267] Modifications may be made to the foregoing without departing from the basic aspects of the invention. Although the invention has been described in substantial detail with reference to one or more specific embodiments, those of skill in the art will recognize that changes may be made to the embodiments specifically disclosed in this application, yet these modifications and improvements are within the scope and spirit of the invention, as set forth in the claims which follow. Also, citation of the above publications or documents is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents. The content of each patent, patent application and other publication and document referenced is incorporated herein by reference in its entirety, including drawings, tables and cited documents.